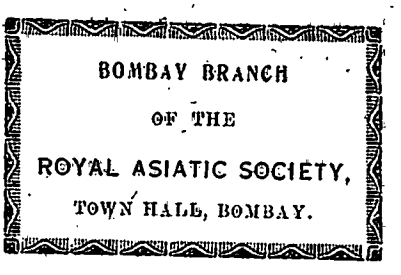
00134335



Digitized with financial assistance from
Government of Maharashtra
on 01 February, 2020

1942
ROYAL ASIATIC SOCIETY

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1941.

134335

Issued under the authority of the Governing Body,
Indian Research Fund Association,
New Delhi,
India.

Price per copy One Rupee.

Obtainable from the Secretary, Governing Body, Indian Research Fund Association, Secretariat, NEW DELHI (India).

00134335

CONTENTS.

		Page
I.	Composition of the Board	i
II.	Composition of Advisory Committees	ii
III.	Meetings held during the year	iii
IV.	Researches carried out during the year	1—119

CHOLERA.

Cholera field enquiry in Bengal under Dr. S. R. Pandit at the All-India Institute of Hygiene and Public Health, Calcutta	1—5
Cholera field enquiry under the Director, King Institute, Guindy, Madras	5—8
Cholera bacteriological enquiry under Dr. G. Panja at the School of Tropical Medicine, Calcutta	8—12
Enquiry on the pathology of cholera including cholera kidney under Dr. D. N. Banerjee at the Carmichael Medical College, Calcutta	12—15

MALARIA.

Malaria Institute of India (Research Section), Kasauli ..	16—18
Malaria transmission enquiry under Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta	18—20
Enquiry on a species of avian malaria under Dr. B. C. Basu at the Imperial Veterinary Research Institute, Mukteswar ..	20—21
Enquiry into the life history of the weeds in the Chilka Lake under Mr. P. Parija, Principal, Ravenshaw College, Cuttack ..	21—25
Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay	25—27

NUTRITION.

Nutrition researches under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, Coonoor	28—33
Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	33—35
Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	35—37
Studies on human nutrition with Indian diets under Dr. K. P. Basu at the Dacca University, Dacca	37—39
Enquiry into calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	39—40

Page

Enquiry into malnutrition of Bengali boys with reference to vitamin A deficiency under Professor N. M. Basu at the Presidency College, Calcutta 41

Enquiry into malnutrition of Bengali boys with reference to vitamins B and C deficiency under Professor N. M. Basu at the Presidency College, Calcutta .. .. .. 42—43

Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lt.-Col. E. W. O'G. Kirwan, I.M.S., Professor of Ophthalmic Surgery, Medical College, Calcutta .. .. 43—44

Enquiry on the nutritive value of fish liver oils obtained from fish along the Bombay sea coast under Professor S. P. Niyogi at the Seth G. S. Medical College, Bombay .. .. 44—46

Studies on the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta .. .. 46—47

Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay .. .. 47—49

Enquiry on the formation functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay .. .. .. 49—51

LEPROSY.

Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta .. .. 52—54

Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras .. .. 55—61

PLAGUE.

Plague researches under the Director, Haffkine Institute, Bombay .. .. 62—64

TUBERCULOSIS.

Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta .. 65—69

Enquiry into the incidence of positive Mantoux reactions and latent tuberculosis as judged by skiagrams in medical students and nurses under Major G. F. Taylor, I.M.S., at the K. E. Medical College, Lahore .. .. 69—70

Epidemiological enquiry on tuberculosis at Saidapet under Dr. R. G. Cochrane, Chief Medical Officer, Lady Willingdon Leprosy Sanatorium, Chingleput, Madras .. .. 70—75

Enquiry into the prevalence of bovine tuberculosis in Vizagapatam under Dr. N. G. Pandalai, Professor of Bacteriology, Medical College, Vizagapatam .. .. .. 75—76

INDIGENOUS DRUGS.

Indigenous drugs enquiry under Bt.-Col. Sir Ram Nath Chopra, I.M.S., (Retd) at the School of Tropical Medicine, Calcutta .. 77—79

Page

Indigenous drugs enquiry under Dr. Khem Singh Grewal at the K. E. Medical College, Lahore .. .. .. 79—80

Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay .. .. 80—81

MATERNAL MORTALITY.

Enquiry into anaemia of women in Assam under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta .. 82—83

Enquiry into the anaemia of pregnancy in Kanara District under the Director of Public Health, Bombay .. .. 83—86

Study of the blood picture in normal pregnancy under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta .. .. 86—87

Pregnancy follow up survey under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta .. 88—91

OTHER RESEARCHES.

Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta .. 92—93

Enquiry into the medical mycology under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta .. .. 93—94

Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta .. .. .. 95—96

Enquiry into filariasis under Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Vizagapatam .. .. 96—97

Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras .. .. 97—99

Protozoal parasites enquiry .. .. .. 99—101

Kala-azar enquiry .. .. 101—102

Enquiry on the algae present in water reservoirs and their effect on purification methods and on public health under the Director, King Institute, Guindy, Madras .. .. 103—105

Enquiry into the isolation of the different constituents of snake venoms with a view to determining their chemical nature under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta .. .. .. 105—106

Enquiry on tetanus under Dr. B. B. Yodh, Lamington Road, Bombay .. .. .. 106—108

Enquiry into vaccine prophylaxis of bacillary dysentery under the Director, Central Research Institute, Kasauli .. 108—110

Immunological studies in allergic states under the Imperial Serologist, Calcutta 110

Haematological and immunological studies on normal blood under the Imperial Serologist, Calcutta .. .. 110—112

Page

Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under Lt.-Col. S. N. Hayes, I.M.S., Medical Superintendent, Lady Willingdon Hospital, Lahore 112–114

Enquiry into the pathology and pathogenesis of splenomegaly under Dr. D. Govinda Reddi at the Medical College, Madras .. 114

Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay .. .. 114—115

Haematological survey of poor class families in and around Lucknow under Dr. V. S. Mangalik at the King George Medical College, Lucknow .. .. 115—118

Leptospirosis enquiry under Dr. M. N. Lahiri at the Haffkine Institute, Bombay .. .. 118—119

V. Reports of advisory committees on

Cholera .. .. 120—123
Malaria .. .. 124—130
Nutrition .. .. 130—133
Plague .. .. 133—134
Maternal mortality .. .. 135—137

VI. Report of the Sub-committee on soya bean appointed by the Nutrition Advisory Committee at its meeting held on the 15th December, 1941. .. .. 138—140

VII. Indian Journal of Medical Research and Indian Medical Research Memoirs .. .. 141—144

VIII. Miscellaneous papers issued under the auspices of the Indian Research Fund Association .. .. .. 145—152

IX. Programme of researches and miscellaneous grants recommended for the financial year 1942-43. .. .. .. 153—156

The Scientific Advisory Board consisted of the following :—

President :

Lieut.-General Sir GORDON JOLLY, K.C.I.E., K.H.S., I.M.S., Director-General, Indian Medical Service.

Members :

Lieut.-Colonel E. COTTER, I.M.S., Public Health Commissioner with the Government of India. *Secretary.*

Major General J. TAYLOR, C.I.E., D.S.O., I.M.S. (Retd.), Director, Central Research Institute, Kasauli.

Bt.-Colonel Sir RAM NATH CHOPRA, C.I.E., I.M.S. (Retd.), Director, School of Tropical Medicine, Calcutta. (1st January to 16th November, 1941).

Dr. L. E. NAPIER, F.R.C.P., Director, School of Tropical Medicine, Calcutta. (17th November to 31st December, 1941).

Lieut.-Colonel S. S. SOKHEY, I.M.S., Director, Haffkine Institute, Parel, Bombay.

Lieut.-Colonel H. E. SHORTT, I.M.S., Director, King Institute, Guindy, Madras. (1st January to 30th June, 1941).

Dr. C. G. PANDIT, Offg. Director, King Institute, Guindy, Madras. (1st July to 26th November, 1941).

Major K. P. MENON, Offg. Director, King Institute, Guindy, Madras. (27th November to 31st December, 1941).

Colonel G. COVELL, C.I.E., I.M.S., Director, Malaria Institute of India, Kasauli.

Dr. JIVRAJ N. MEHTA, Dean, Seth G. S. Medical College, Bombay.

Dr. V. R. KHANOLKAR, Seth G. S. Medical College, Bombay.

Dr. G. SANKARAN, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Coopted members.

Dr. W. R. AYKROYD, Director, Nutrition Research, I. R. F. A., Coonoor.

Dr. JEAN M. ORKNEY, W.M.S., Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi.

Composition of Advisory Committees for the year 1942.

1. *Cholera.*

Major General J. TAYLOR, (*Chairman*).
Lt.-Col. L. A. P. ANDERSON.
Dr. L. E. NAPIER.
Dr. K. C. K. E. RAJA.
Colonel H. E. SHORTT.
Dr. K. V. VENKATRAMAN.
Dr. S. R. PANDIT, (*Secretary*).

2. *Malaria.*

Colonel G. COVELL, (*Chairman*).
Rai Bahadur Dr. A. C. BANERJEA.
Bt.-Col. Sir RAM NATH CHOPRA.
Dr. K. A. GANDHI.
Dr. D. K. VISWANATHAN.
Major JASWANT SINGH, (*Secretary*).
Dr. G. C. RAMSAY
Dr. PAUL RUSSELL.
Mr. R, SENIOR WHITE.
} Have been asked to attend as visitors.

3. *Maternal Mortality.*

Major General W. C. PATON, (*Chairman*).
Dr. L. GHOSH.
Lt.-Col. S. N. HAYES.
Dr. J. JHIRAD.
Dr. L. E. NAPIER.
Dr. M. I. NEAL EDWARDS.
Dr. N. A. PURANDARE.
Dr. J. M. ORKNEY, (*Secretary*).

4. *Nutrition.*

Lt.-Col. B. COTTER, (*Chairman*).
Dr. K. P. BASU.
Dr. N. M. BASU.
Dr. W. BURNS.
Dr. T. B. GREGORY.
Dr. B. C. GUHA.
Dr. V. N. PATWARDHAN.
Dr. G. SANKARAN.
Mr. J. SARGENT.
Mr. F. WARE.
Dr. W. R. AYKROYD, (*Secretary*).

5. *Plague.*

Lt.-Col. S. S. SOKHEY, (*Chairman*).
Lt.-Col. L. A. P. ANDERSON.
Dr. V. R. KHANOLKAR.
Colonel H. E. SHORTT.
Major General J. TAYLOR, (*Secretary*).

The Board held two Meetings during the year, the first on the 15th and the second on the 16th and 17th December, 1941.

At its meeting on the 15th December, 1941, the Board confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers, and also decided on the cooption of experts for its meeting on the 16th and 17th December, 1941. On these dates the Board considered in detail the programme of research for the year 1942-43. The programme of researches recommended by the Board is given on pages 153–156 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1941.

The researches, which were carried out during the year under report, and which were recommended by the Board at its meeting on the 19th December, 1940, were approved by the Governing Body of the Association at their meeting held in the Imperial Secretariat, New Delhi, on the 8th March, 1941.

CHOLERA

1. Cholera field enquiry in Bengal under Dr. S. R. Pandit at the All-India Institute of Hygiene and Public Health, Calcutta.

Major W. D. B. Read, I.M.S., continued to be associated with this enquiry up to the end of the first quarter of the year, when his services were placed at the disposal of the Army department. The field work which was confined to the first quarter consisted of water examinations in the district of 24-Parganas, the rest of the year being spent in the examination of cholera stools from the Campbell Hospital, of waters in the city and outskirts of Calcutta and in laboratory studies.

WATER EXAMINATIONS.

(*a*) *In Sind*—These examinations were carried out from the last week of October to the third week of December, 1940, in the cholera-free district of Karachi; the earlier results were reported last year. The examination of a total of 552 samples from 206 water sources showed that 18 sources were positive, the El Tor type being isolated. All the strains were of the intermediate (Hikojima) type except one which was Inaba. A tank in Tatta was positive over a period of 41 days, all the eight specimens collected during the period being positive. Two *jhils* were positive over 35 and 41 days respectively, 4 out of 8 samples in the former and 3 out of 4 in the latter being positive. In the remaining positive sources isolations could be effected only once.

(*b*) 24-*Parganas*—A field unit worked in this district during March 1941, when cholera was prevalent. The improved method of filtration of water which was tried out first at Patna last year and then in Sind was employed in this investigation also.

One hundred and eightynine samples from 145 water sources were examined from six villages where cholera cases were occurring or had recently occurred and 195 samples from 5 villages with no cholera. The latter were all negative. In the former, 15 out of 189 samples (14 out of 145 sources) were positive, *V. cholerae* being isolated. Samples were repeated from several of the sources and invariably from positive sources. Only one source, a khal, was positive at the second examination—on the eleventh day but negative at the third examination on the nineteenth day. The remaining 13 positive sources were all tanks. All the positive sources were directly connected with cases occurring in the vicinity. The El Tor type was not isolated from any of the sources examined.

(*c*) *Calcutta*—During the period 18.8.41 to 14.11.41, *i.e.* in the inter-epidemic period of Calcutta, 364 samples were collected from the city outskirts, Only one, from a *jhil*, was positive, the organism isolated being haemolytic Ogawa type. A second examination made 4 days after and two subsequent examinations from the same source gave negative results.

During the epidemic 45 samples from the Hoogly river were examined for Ag vibrio but with negative results. Since then, an examination of 49 more samples from the river has revealed the presence of *V. cholerae*, Ogawa type, in one sample collected on 25.11.41 . During the period that these latter examinations were made, only stray cases of cholera occurred in the city.

The experience of the endemic Khulna area over a year had shown that the El Tor type of vibrio could be isolated from about 3% of water sources and that there was a seasonal variation, the largest number of isolations being made in the rainy season in July and August. In the Sind area, where cholera had been unknown for 10 years, the El Tor vibrio was isolated from 8.7% of sources examined in early winter. In Calcutta, examinations of tank waters etc. following a severe epidemic of cholera have revealed only one source positive for the El Tor type. The relationship of this type to the epidemiological prevalence of cholera seems to be quite insignificant.

Cholera stools.

Examination of stools from cholera patients admitted to the wards of the Campbell Hospital was undertaken primarily to determine if the El Tor type could be isolated at all and if so at which particular phase of the epidemic as well as to ascertain whether a change to the haemolytic type occurred in the gut of the convalescing case. When the work was commenced in the first week of April there was a high admission rate to the wards, and this was kept up till the first week of July averaging 270 admissions per week after which there was a fall to 16 admissions per week, a rate which has been kept up till the date of this report. The death-rate in hospital patients in both these periods was practically the same—about 10%. Examinations were continued till the end of September. Specimens were collected usually within 24 hours after admission and were plated on agar, Aronson and the modified solid medium of Wilson and Reilly both directly and after enrichment in modified Wilson-Blair fluid medium. Only one specimen was collected from each case. At least three Ag colonies were picked up from the plates and examined for their haemolytic power in blood broth ; a larger number of colonies were invariably examined from cases occurring in the declining phase of the epidemic and the inter-epidemic period. During the epidemic phase 308 cases and thereafter 122 cases were examined, of which 251 (81%) and 33 (27%) respectively were positive. In none of these was the El Tor type vibrio encountered. Only three cases from whom *V. cholerae* had been isolated during the attack, could be followed up in their convalescene but the Ag vibrio could not be isolated from any of them.

It was found that during the epidemic phase marked by a high admission rate to the hospital, the percentage of isolation was 81 ; at its height 92% proved positive (95 out of 103). The Inaba type was isolated in 27% of the positives, the Ogawa type in 66% and both the types in 7%. In the succeeding phase with a low admission rate, the percentage of isolations was 27, the Inaba and Ogawa types being found in 36 and 64% respectively. During these examinations the inagglutinable vibrio was not infrequently encountered but not more frequently in negative stools than in positive stools.

A comparison of direct plating and the enrichment methods showed that 96% of the positives were detected by the former and 89% by the latter. So far as stools from patients were concerned therefore direct plating was superior to the other method. It had also the advantage that the results were available 24 hours earlier.

Laboratory studies.

Haemolysis—This work, the earlier results of which were communicated in last year's report, was completed during the present year. The strains studied, 62 in all, included case and water strains isolated in Bengal in the presence of cholera, El Tor type strains isolated from Indian waters, El Tor type strains from waters and cases from Celebes (kindly sent by Dr. de Moor from Macassar) and some classical El Tors. The organisms were grown in isotonic Douglas broth, both aerobically and under restricted oxygen supply for periods varying from one to fifteen days and the resulting growths tested on washed goat red cells by continuous incubation at 37° and 12° C. in the presence of air and under restricted oxygen supply (vaseline seal and Mac Intosh and Fildes' jar). Both 2·5 and 0·5% concentrations of cells were employed. The strains were also grown in blood broth of the same blood concentration and also on 10% blood agar under aerobic and anaerobic conditions. The results showed that the Greig test was supported in some degree by all methods of test. The strains could be divided into 2 groups, the "early haemolytic" and "late haemolytic", corresponding to Greig positive and Greig negative organisms. In the former, haemolysis was usually complete in a few minutes to 24 hours, was not affected by performing the test at 12° C. or under restricted oxygen tension and the production of haemolysin was not affected by the exclusion of oxygen. In the latter group, haemolysis was usually partial, it hardly occurred in 24 hours and was abolished by performing the test at 12° C. or under restricted oxygen tension. Anti-haemolytic sera prepared from Greig positive organisms while having a specific neutralising effect on the haemolysin of the early haemolytic group, had no similar effect on the late haemolytic group. Formalin-killed cultures of either group had their distinctive effect though less marked than in live cultures. The haemolysin of the early haemolytic group could be filtered through an L_3 candle but not that of the other group. It was concluded that the "early" and "late" haemolysins were very likely identical with the haemolytic and haemodigestive ferments described by van Loghem working with solid blood media.

2. *Growth of filtrates of cholera stools on the chorioallantoic membrane*—Stools of 6 cholera patients, proved bacteriologically positive, collected within 24 hours after the onset of symptoms were filtered through L_3 candles and the filtrates planted on the chorio-allantoic membrane of the developing chick 10-14 days old. From three of the cases, blood sera collected at the same time, were also similarly passaged. The eggs were opened after 3-6 days. No macroscopic lesions could be seen in any nor in 3 sub-passages conducted with three of the stool filtrates. It was concluded that if a filtrable virus was at all associated with cholera, its presence could not be detected by the methods employed.

3. *Bacteriological examination of bile and duodenal contents from post-mortem cases*—These examinations in post-mortems from the Police Morgue were commenced towards the end of June in order to see if the carrier-state existed and could be detected by this method. Of the 88 cases examined, there was none suspected of dying from cholera. In the majority of the cases the cause of death was accident. The Ag vibrio was not isolated in any. An Nagg vibrio, Heiberg I, was isolated from bile in one case and in 5 others inagglutinable vibrios were isolated from duodenal contents. The bile was found to be sterile in only 16 cases, one or more kinds of organisms being usually present in the rest. The possibility of an invasion of the gall bladder by organisms from the gut as a post-mortem occurrence, is to be considered.

PH determination showed that the pH of bile was usually below 7·8, the figure mentioned in books. In only two instances out of 43 where estimations were made, was the pH at this figure, the lowest recorded being 5·8. The median average was 6·6. In the same number of pH estimations of duodenal contents, the median average was 6·8, the lowest pH recorded being 5·4 and the highest 7·6.

4. *The solid medium of Wilson and Reilly*—This medium prepared according to the authors' formula, but substituting Liq. bismuthi for bismuth citrate scales, was found to be very inhibitory and had to be modified. As thus altered, the formula in routine use was—2·5% nutrient agar, prepared from papain broth, pH 8·8, 100 c.c., 20% sodium sulphite 4·8 c.c., Liq. bismuthi 0·16 c.c., absolute alcohol 0·2 c.c., and mannose solution equivalent to 0·1 gr. mannose. It was found to inhibit the El Tor vibrio and many inagglutinable vibrios, though not all. It had also an inhibitory effect on many organisms other than vibrios and was found to be superior to Aronson. This medium was in routine use along with agar and Aronson plates in all examinations.

5. *Differential agglutination of V. cholerae and El Tor.*—Dr. de Moor had found that by employing a concentration of 0·3% Na_2CO_3 in saline dilutions of sera, the El Tor type could be distinguished from *V. cholera*, agglutination taking place in the case of the former but not in the latter. This was confirmed and the method was found to be a useful basis of differentiation. It was found that 0·3 to 0·4% concentration was the optimum ; a certain amount of lysis of the vibrio however took place. At lower concentration both types were agglutinated and at a concentration of 0·9% agglutination did not result with either type of organism. Washings in distilled water or saline of agar growths were more suitable than formalinised suspensions or heated suspensions and " O " sera more suitable than " H plus O " sera. For the test incubation at 37°C. in the incubator was more suitable than at 55°C. in the water bath. The tests were best performed in round bottomed tubes using fresh thick suspensions in distilled water, of which a drop or two was added to the alkalinised serum dilutions.

It was found that acid agglutination could not distinguish the El Tor from the cholera vibrio. With sodium lactate and lactic acid mixtures giving a pH range of 2·2 to 4·7 and using killed and live vibrio suspensions in distilled water, both types agglutinated and a grouping of the two types was not found to be possible.

6. *Action of phage N*—This type of cholera phage described by Pasricha *et al* (1941) had been claimed to have a differential action on *V. cholerae*, 10 El Tors and a large number of Nagg vibrios tested by them being found resistent to it. Its action on a large number of haemolytic and non-haemolytic vibrios was tested and with some exceptions, it was found to act on both types alike. It was also found that its action on growths from different colonies from the same cholera stool plate was not always the same. As a means of differentiating the El Tor from *V. cholerae*, this phage type is of little use.

7. *Flagellar stain*—Flagellar staining of a large number of El Tor strains from Indian waters, all the Celebes strains in the enquiry's collection and some classical El Tors showed that they were all monotrichate, differing in no way from *V. cholerae.*

8. *Experimental work on the selective medium*—Throughout these three years, the formula of the modified Wilson-Blair fluid medium as laid down by Read (1938) had been employed with the exception that the concentration of Liq. bismuthi was reduced to a third. Some experimental work was done

in order to enable simplification of the formula and particularly to determine if the use of mannose was necessary. Working with stool suspensions inoculated with minimal doses of *V. cholerae* and larger doses of mannose fermenting and non-fermenting inagglutinable vibrios, it was found that the selective action of the medium was independent of mannose. Glucose or mannite could be substituted in its place though they were less efficient than mannose and without any sugar, results were as good as with mannose and often better. Bismuth and sulphite are the most important ingredients of the medium in this differential action and alcohol and perchloride are of secondary importance having perhaps an inhibitory effect on spreaders. There was an indication in some of the experiments that 0·2 to 0·4 c.c. of alcohol aided isolation ; larger concentrations were inhibitory. As regards bismuth, three-fold and five-fold concentrations, 0·12 and 0·2 c.c. respectively, gave better results than 0·04 c.c., the routine dose ; higher concentrations were inhibitory.

In the water experiments, any loss of the added organisms in the process of filtration or by death was overcome by filtering the waters first, crushing the filter papers in the medium and then adding the Ag and Nagg organisms. The results showed that the full formula gave the best isolations, that isolations were poorer in the absence of a sugar and mannose was better than glucose or mannite. Doubling the dose of bismuth gave better results but a greater concentration was inhibitory. In general, as compared to stools, isolations from water were much more difficult.

A comparison of freshly isolated and old case strains showed that there was little difference in the ease of their isolation from stools and water.

With waters inoculated with El Tor and *V. cholerae* in comparatively moderate doses, it was found that both the types were eliminated with the same quickness. The explanation of the persistent isolation of El Tor over long periods from certain waters as in Sind is possibly to be sought in the constant re-infection of the water from some unknown source.

2. Cholera field enquiry under the Director, King Institute, Guindy, Madras.

The work of the enquiry was carried out in the field laboratory at Tanjore during the first quarter and subsequently at the King Institute, Guindy. The change of headquarters was made in accordance with the recommendations of the Cholera Advisory Committee of 1940 owing to the absence of cholera in the selected field area.

The field work done during the year included an investigation of an isolated outbreak of cholera in two villages of Villupuram Taluk in a region where cholera was absent for several months previously ; an investigation of cholera in Ampalam village, Vizagapatam district, where cholera had been prevalent in an epidemic form for over a year and a re-examination of the water sources near Negapatam in the continued absence of cholera.

The laboratory investigations carried out during the period included further examination of the usefulness of the preserving medium in the transmission of specimens for the isolation of *V. cholerae* described in the previous year's report ; a re-examination of the haemolytic strains isolated in previous years and of the methods of demonstrating haemolysis in vibrio strains ; a study of the methods of preparation of mannose solution for bacteriological

use from ivory nut and an examination of 347 strains of *V. cholerae* isolated from specimens received at the Institute and by the field units.

The monthly figures for cholera mortality in the several districts of Madras Presidency during the fifteen years (1926-1940) succeeding the period of A. J. H. Russell's investigation (1896-1925) were collected and studied in collaboration with Dr. K. C. K. E. Raja with a view to determining the real endemic status of Tanjore district.

Field work.

1. In February, a limited outbreak of cholera occurred in two villages of the Villupuram Taluk with a total of 27 attacks and 14 deaths. The cases were restricted to one hamlet in the first village, Pillur, leaving the main village and the remaining hamlets entirely free. Nineteen cases were recorded in a population of 589. Fiftythree specimens were obtained from 13 cases and *V. cholerae* was isolated from 12. In 196 specimens from 61 contacts, *V. cholerae* was isolated from 4. Subsequent examinations of these contacts were negative. In the case of the other village, Kappur, two cases occurred in the main village (polpuation 889) and 6 in a hamlet (population 450). Twelve specimens were obtained from 5 cases and *V. cholerae* recovered from 4. In 134 specimens from 48 contacts of this group of cases, *V. cholerae* was recovered from 2. *V. cholerae* could not be recovered from any of 51 specimens from 18 persons resident in the adjoining hamlet with a population of 95, where there were no cases. Of the 6 positive contacts, 4 had been inoculated 3 to 9 days previously and the other 2 were unprotected. All the strains, from both cases and contacts, with one exception were of the *OGAWA* type and non-haemolytic. One strain from a contact (resident of a house adjoining the one where there was a fatal case) was *INABA*, also non-haemolytic. Examination of water sources was interfered with by vigorous chlorination that was being done. Eleven samples obtained from some of the sources were all negative.

The village of Pillur was revisited in August, six months after the previous outbreak. Two hundred and thirtyseven specimens were obtained from 49 members of 11 families in whom *V. cholerae* was demonstrated during the previous visit. No *V. cholerae* could be isolated from any of the specimens.

2. In Ampalam village (Vizagapatam district) there had been 29 attacks and 11 deaths from cholera in a community of 450 people, 382 of whom were inoculated (after the onset of the outbreak). By the time the investigation could be started, the outbreak had subsided and no further cases occurred during the period. All persons resident in houses from which cholera had been reported in the previous week were included in this survey. A total of 395 specimens were obtained from 86 members of 15 families. In 5 houses, the originally reported cases were dead and there was no active source of infection within the house. In repeated examinations of stools, no *V. cholerae* could be isolated from any of the 10 recovered cases. Five specimens from contacts proved positive, one each from 3 who were not inoculated and two from the fourth who was inoculated 10 days previously. The ages of the 4 positive contacts were 3, 1, 3 and 12 years. Several subsequent examinations of these positive contacts failed to show agglutinable vibrios.

3. A further set of 110 specimens was obtained from 21 members of 4 houses in Tilaru in the same district. Two were positive, one from a case 3 days after the onset and the other from a contact during the active period of the case. The positive contact (age 35) had been inoculated only the previous day, and she continued to remain well during subsequent weeks. All these strains were *OGAWA* (non-haemolytic).

In these examinations, advantage was taken of the KCl-buffer preservative which enabled large numbers of specimens to be examined conveniently in the laboratory. The presence of temporary carriers among contacts observed in this investigation suggests the need for further enquiry on their incidence in relation to the prophylactic inoculation of the individual and the intensity of the inoculation campaign in the community, these will be taken up during the coming year.

4. The observations made during the previous year on the occurrence of *V. El Tor* in natural water sources in Tanjore district were collated and published in the Indian Journal of Medical Research, Vol. 29, 3, July 1941.

During October 1941, 132 specimens of water were obtained from the 33 tanks near Negapatam which had been repeatedly examined in the previous year. Eight specimens (from 8 sources) yield agglutinable vibrios. All these were haemolytic and of the *OGAWA* type. These 8 sources are different from those previously recorded positive. In the examination of these specimens, the filtration was done in the field and the filter paper transmitted by mail to the King Institute where they were cultured in Read's modification of the Wilson & Blair medium with the further modification that buffered KCl-boric acid saline was substituted for normal saline. This made it possible to do away with the readjustment of the pH 3-5 hours after inoculation.

Laboratory work.

1. The preserving medium was used extensively during the year and found to be adequate. A note on the subject has been published in the Indian Journal of Medical Research Vol. 29, 4, October, 1941.

Experiments were undertaken to determine the limit of tolerance to boric acid of *V. cholerae*, *Bact. coli* and *Bact. aerogenes*. These showed that, with a small inoculum of approximately 100 organisms, *V. cholerae* could multiply in media containing as much as 1·2% boric acid, whereas *coli* and *aerogenes* were inhibited by as little as 0·3% boric acid. With heavy inocula, this differential inhibition was not noticeable. One c.c. of 18-hour broth culture as inoculum completely overcomes the inhibitory effect of even 2·1% boric acid.

2. *Haemolysis*—The use of 24-hour cultures in isotonic Douglas' broth has enabled consistent results to be obtained. The results obtained on superimposed goat blood agar follow closely those in Douglas' broth. Storing the plates for a period of 24 hours in the ice-box (subsequent to 24 hours' incubation at 37°C) brings out the difference between haemolytic and non-haemolytic strains more clearly. A characteristic concentration of the red cells and an intensification of the red colour occurs outside the clear transparent halo round colonies of haemolytic strains.

3. *Preparation of mannose*—Difficulties were met with in the preparation of mannose solution from ivory nut by previously described methods owing to the high colour of the resulting product. Suitable decolourising charcoal could not be obtained to overcome this difficulty. It was found that by allowing the hydrolysis to proceed at 45°C over 8 to 10 days, a clear solution, could be obtained which was quite suitable. By adjusting the quantity of distilled water used in diluting the acid solution, prior to neutralisation with barium hydroxide, mannose could be obtained in sufficient concentration to meet the needs. The yield in terms of the washed nut compares favourably with those obtained by other methods. The amount of mannose formed on different days is as follows :—

Day	..	1	2	3	4	5	6	7	8	9	10
Mannose. (% of ivory nut).	..	10	18	27	31	35	36	39	40	40	39

4. *Distribution of serological types of V. cholerae*—A total of 347 strains of *V. cholerae* (including 186 isolated towards the end of last year) were examined. Three hundred of these were derived from the Northern Circars, 20 from the Ceded districts, 23 from South Arcot and the remaining 4 from sporadic cases in the central districts. All the strains were non-haemolytic. Of the total strains 342 were *OGAWA*, 4 *INABA* and 1 *HIKOJEMA* (or the intermediate type). One *INABA* was derived from a healthy contact in South Arcot, two *INABAS* and one *HIKOJEMA* from cases in East Godavari district and the last *INABA* culture from a case in Guntur. *OGAWA* has continued to be the predominant type during the year.

5. The results of the analysis of the cholera mortality figures for the several districts of the Province appear to corroborate the findings of Russell based on the figures for the previous 30 years that the frequency of cholera infection is more in the districts of Tanjore, Trichinopoly and South Arcot than in the other districts of the Province. But the continuity of infection is being maintained in Tanjore because the seasonal incidence of the disease varies in different parts of the district, with the result that by the time cholera disappears in one area it starts in another. This seems to be a spurious form of endemicity. The fact that these three types of areas have been brought together to form a single district is purely due to administrative convenience. If, on the other hand, the deltaic areas of Tanjore, Trichinopoly and South Arcot had constituted a district, the recorded vital statistics for this area would have shown that the incidence of cholera was mainly confined to the winter months. The district would not have been then considered as an endemic area. It cannot, therefore, be accepted that the endemic status of Tanjore has been established.

3. Cholera bacteriological enquiry under Dr. G. Panja at the School of Tropical Medicine, Calcutta.

1. *With human plasma*—The trial was given on a few cases. Blood pressure, specific gravity of blood, haemotocrat readings etc. were taken. No opinion can be expressed until further trial is given on a large number of cases.

2. *With pyrogen-free saline*—Out of 30 cases treated with pyrogen-free saline, 20 cases had neither rigor nor any rise of rectal temperature, whereas out of 18 cases treated with ordinarily prepared hospital saline 12 cases had severe rigor and high rectal temperature and 5 cases had rigor only. Pyrogen-free saline was administered with safety in cases showing even high rectal temperature. Out of 11 cases so treated, 6 cases had neither rise of temperature nor rigor and 5 cases had fall of the high temperature.

3. *With dyes per mouth*—The bacteriostatic and bactericidal actions of high dilutions of brilliant green, malachite green and acriflavin on cultures of vibrios were demonstrated in vitro. When brilliant green was added to 25 samples of cholera stool in a dilution of 1 in 5,000, vibrios could not be isolated from 19, i.e., 76% of the samples. The dye dilution (1 in 5,000) was given to cholera patients in ½ oz. doses every 2 hours and daily examination of stools for vibrios was carried out. No opinion can be expressed until a further trial is made on a large number of cases during the next epidemic.

The cholera vomit.

The pH of the vomits varied from 2·4 to 8·4. The commonest pH was from 6·0 to 7·0. Vibrios were isolated from about 48% of the samples of the vomits. For isolation direct plating was more successful than the enrichment method. Only one vomit was examined from each case. If more vomits were examined from each case the percentage of isolation would have been higher. Both Ag and Nag vibrios were isolated from some samples. Ag vibrios were serologically identical with those isolated from stools. The sodium chloride content of the vomits varied from 66 to 821 mgm. per 100 c.c. of vomit. There was no correlation between pH and sodichloride content.

Isolation of vibrios from cholera stool.

A new method of isolation—When a sample of cholera stool, mixed with a little peptone water, is put into a L_3 candle and then the candle is placed in 0·08% boric peptone water (pH 9·0) contained in a wider test tube in such a way that the unglazed part of the candle is covered by the surrounding peptone water and the whole thing is incubated for 18-48 hours, a pure or almost pure culture of vibrios is often obtained. Boric acid acts as an inhibitory agent to coliform organisms. Out of 45 samples of cholera stool examined during the declining period of the cholera epidemic, vibrios were isolated from about 44% of the samples on bile salt agar and more easily from about 87% of the samples by the candle-boric-peptone-water method. Modified Wilson Blair's medium kindly supplied by the Cholera Field Inquiry was used in the candles instead of boric peptone water in 11 samples of stool. Vibrios were isolated from all the 11 samples by the candle-boric-peptone-water method but from 7 samples by the substitute method. Soil, natural waters, faeces, broth cultures of coli, proteus, faecalis, pyocyanea, typhosum, subtilis etc. were artificially mixed with a small number of vibrios and from such mixtures, pure cultures of vibrios were often obtained by the new method but by direct plating the result was often negative. The method was not suitable for differential isolation of Ag and Nag vibrios.

Vibrio faecalis—The organism was isolated in large numbers on plate cultures of several samples of cholera stool and it appeared that this might be the cause of cholera but the organism was found to be non-pathogenic to guinea pigs and no antibody was demonstrable in the cholera patients' blood against this organism.

Incidence of serological types of vibrios in the cholera epidemic of 1941.

Number of cases examined	148
Vibrios positive in cases	105

Inaba type only	13	Inaba & Ogawa both	4
Ogawa type only	50	Inaba and Nag	3
Nag type only	15	Ogawa and Nag	19
		Inaba, Ogawa and Nag	1

Cholera patients' blood.

1. *Agglutinin*—Felix method of agglutination with live cultures was found more sensitive than Dreyer's method with killed cultures. So the former method was adopted for the test. Agglutinins for Inaba, Ogawa and Basra

were found in about 63% of the normal sera in Calcutta and the titre of agglutination was commonly 1 in 10. Agglutinins were present in about 91% of cholera case sera and the titre was commonly 1 in 20. Specificity regarding causative types was not uniformly found. In another series of cholera cases 71-74% showed agglutinins for vibrios. In cases where Ag and Nag types were both isolated, agglutinins were present sometimes for both and sometimes for Ag only. These results indicate that agglutinins are of no value in diagonsing cholera cases in an endemic area.

2. *Bacteriolysin*—An effort was made to find this antibody in the blood of cholera cases by modified Pfeiffer's test in vivo hitherto untried by any. The antibody was found in normal sera. In cholera case sera 33% were positive with homologous vibrios. Specificity regarding the causative type was not found in the majority of cases although, in a few cases a striking specificity was seen not only in bacteriolysis but also in the ultimate survival of the guinea pigs tested. Bacteriolysins were found even in the early stage of the disease.

3. *Complement fixing antibody*—Only 11 sera were tested with negative results in most of them. There was no specificity in the positive sera.

4. *Takata-Ara-Reaction*—This was positive in only 2 out of 30 case sera tested.

O-ANTIGEN ON LITHIUM CHLORIDE AGAR AS REPORTED BY RUSSO (CANIO).

This was tried without any success. Inaba and Bact. typhosum maintained their motility in ½ and 1% lithium chloride agar slopes and semi-solid agar deeps when subcultured for 8 consecutive days.

ARTIFICIAL MIXTURES OF AG AND NAG VIBRIOS.

A mixture of Ag and Nag vibrios was kept on solid as well as in liquid media. Subculture at weekly intervals up till the end of the 4th week showed an almost complete annihilation of Ag vibrios and survival of the Nags. This experiment may largely explain why Nag vibrios are so frequently met with during the latter part of the disease or during convalescence or during the inter-epidemic periods.

DIFFERENTIATION BETWEEN INABA, OGAWA AND EL TOR VIBRIOS.

A trial was given with the following tests:—(*i*) Inulin fermentation, (*ii*) bile solubility and solubility in 22 different substances (acids, salts, glycocoll, hydrogen peroxide etc.), (*iii*) growth in Koser's citrate solution, (*iv*) colony characters on haemoglobin media, (*v*) mass staining of colonies with 11 different dyes, and (*vi*) agglutination with alkalinised (0·3% Na_3 CO_2 in normal saline) sera in Felix tubes but without any success. In alkalinised high titre sera, agglutination was clear cut with El Tor strains and less so with Inaba and Ogawa but the titre of agglutination was the same.

LIVE VIRULENT VIBRIOS INJECTED INTO MAN AND ANIMALS.

Live virulent vibrios when injected subcutaneously or intraperitoneally into guinea pigs were recovered from the peripheral blood every 5 minutes up to 2 hours tested. None was found in the stool, but the gut showed catarrh and congestion. After a dose of 20,000 millions of live vibrios injected subcutaneously into man no vibrios were found in the peripheral blood as well as in the stool in two cases, although there was marked local reaction associated with pyrexia.

Effect of sulphanilamide compounds.

M. B. 693 (1-2%) and soluceptasin were tested in vitro. These are not bacteriostatic. Soluseptasin delays the haemolytic action of El Tor and Nag vibrios for 16-20 hours.

Study of the cholera case Nag vibrios.

1. *Frequency*—Nag types were more frequently isolated during the latter part of illness although in many cases Nag types only were found. Some vibrios when first isolated were labelled as Nag but on repeated sub-cultures found to be Ag.

2. *Serological*—Heterogeneous character has been found by Taylor and Pandit amongst Nag vibrios isolated from various sources. This is corroborated by us. Over and above by agglutinating different strains isolated from the same case with a serum raised against one strain heterogeneity has also been found. The following table shows the results :—

Sera raised with case strain.	Own strains.			Nag strains isolated from other cases.			Standard.	
	Number of strains isolated on different days.	+	—	Number of strains	+	—	Inaba	Ogawa
151	10	6	4	11	9	2	–ve	–ve
153	4	3	1	11	2	9	–ve	–ve
163	6	2	4	11	3	8	–ve	–ve

3. *Heiberg's classification*—Homogeneous and heterogeneous groups both found in the same case.

Number of cholera cases examined	.. ..	38
Number of Nag strains examined	.. ..	125
Heiberg's Group I .. 40		
,, ,, II .. 79	.. ..	125
,, ,, V .. 6		
Homogeneous strains isolated from	.. ..	11 cases
Heterogeneous (dual groups only) from	.. ..	8 cases

4. *Haemolysis test*—All Nag strains are not haemolytic. Some are non-haemolytic. In the same case of cholera, haemolytic and non-haemolytic strains were isolated just as heterogeneous antigenic types were isolated. Some haemolytic Nag vibrios showed production of haemolysin in liquid medium when cultured with goat's blood aerobically and anaerobically but failed to show haemolysin when cultured without blood aerobically and there was no beta-type of haemolysis in solid blood agar plates. This may be due to the fact that either the haemolysin was oxygen-sensitive or it was inactivated by some metabolic product of the vibrios. The following table shows the result of the haemolysis test :—

	Aerobic		Anaerobic		Deep blood agar plate.
	Nutrient broth		Nutrient broth		
	With blood	Without blood	With blood	Without blood	
141 a (Nag)	H	H	H	H	Beta type
135 bz (Nag)	H	O	H	H	Alpha type

5. *Effect of dyes*—Some Nag vibrios are sensitive to green dyes but the majority are non-sensitive. Brilliant or malachite green in a dilution of 1 in 50,000 to 100,000 inhibits as well as kills some Nag vibrios.

EFFECT OF DYES ON THE VIBRIOS.

1. *Bacteriostatic experiment on dye-agar plates.*

Brilliant green	1 in 100,000 to 250,000	inhibits Inaba, Ogawa and also Coli and Flexner.
Crystal violet	1 in 100,000	,, ,, ,, but not Coli & Flexner.
Acriflavin	1 in 100,000	,, ,, ,, ,, ,, ,,
Gentian violet Crystal violet Methyl violet Methylene blue	1 in 50,000	,, ,, ,,
Malachite green	1 in 50,000	,, ,, ,,
Thionin	1 in 25,000	,, ,, ,,
Safranin Fuchsin	1 in 5,000	,, ,, ,,

2. BACTERICIDAL EXPERIMENT WITH 10 C.C. OF YOUNG BROTH CULTURE.

Brilliant and malachite green in a dilution of 1 in 100,000 kill freshly isolated Inaba and Ogawa vibrios in 2-20 hours but are apparently harmless to Bact. coli, aerogenes, typhosum, flexneri and vibrio faecalis. The same dilution of acriflavin, crystal violet and gentian violet have no bactericidal effect on the above organisms.

POTENCY OF CHOLERA VACCINE.

One batch of commercial cholera vaccine was found inagglutinable with Kasauli serum. Control Kasauli vaccine was agglutinable. The commercial vaccine was highly agglutinogenic in rabbits and protective in guinea pigs. The vaccine did not contain any Gram-positive organisms and spore-formers. It is true that agglutinability is an indicator of potency but potency may not always be determined by agglutinability. The above finding indicates that an inagglutinable vaccine may still be highly potent. This is only one isolated example.

4. Enquiry on the pathology of cholera including cholera kidney under Dr. D. N. Banerjee at the Carmichael Medical College, Calcutta.

Work was carried out under the following heads.—

1. Pathology of the alimentary tract in cholera.

2. Cytological study of cholera evacuation.
3. Role of the presence of spirillum and spirochaetes in the alimentary tract.
4. Pathology of pulmonary oedema in cholera.
5. Study of bone marrow and suprarenal in cholera.
6. Concentrated saline in the treatment of cholera.

1. Pathology of the alimentary tract in cholera.

(*a*) *The site of the lesion*—Cultures have been made from material obtained immediately after death from different parts of the alimentary tract. It has been observed that the presence of the vibrio is almost of equal frequency and number whether the material was obtained from small intestine or large intestine. The intestinal lesion, on the other hand, is exclusively in the small intestine. With a view to finding out how the vibrios produce the lesion a series of histological examinations has been done of different parts of the alimentary tract from the stomach to the rectum. This material is still under study.

(*b*) *Histological examination for demonstration of the vibrio in the tissue*—Goodpasteur's method has been adopted. (*i*) It has been observed that the vibrios are found in the wall of the small intestine and only rarely in the wall of the large intestine. (*ii*) The vibrios are present inside the mucosa, submucosa and also at the base of the Lieberkuhn's follicles and only in very rare instances in the subserous layer.

Further work is in progress to investigate the cause why the small intestine only is affected by the invasion of vibrios inside the mucous membrane and not the large intestine although the lumen of both the small and the large intestines contain equal numbers of the vibrio.

(*c*) *The nature of intestinal lesion*—It is generally believed that the lesion of the cholera intestine is non-inflammatory in nature. Peroxydase reaction of the histological sections shows that a good number of areas in the small intestine show evidence of collection of polymorphonuclear cells, and other evidences of imflammation. Necrosis and desquammation of the epithelium is almost widespread. The cause of desquammation is not known, nor is it known why the small intestine should alone be affected and not the large intestine. It is generally believed that the cholera toxin present in the lumen is responsible for the same and it has been suggested that desquammation is caused by—virulent enzymes secreted by the vibrio.

With a view to solving this problem a study has been made on the enzymes present in the cholera stool. It has been found that the enzymes sucrase, lactase and maltase are not generally present except in very few samples, while erepsin is not found at all.

Further work is in progress regarding the effect of the supposed necrotic enzyme, if there be any, on the normal human intestine removed immediately after death from other diseases and also that of animals. This is being done by the process of chromolysis elaborated by Unna. The writer has already worked on this method for histological determination of the chemistry of cells (Cal. Med. Jour. Feb. 1930), and chromolytic determination of the chemistry of haemoproteus columbae (Cal. Med. Jour. April, 1931).

(*d*) *Study in progress*—(*i*) The Payer's patches and solitary follicles and also the mesenteric glands invariably show considerable hyperplasia and necrosis. A detailed histological study of lymphoid elements as well as of the thymus which is also found to be constantly enlarged is in progress. (*ii*) A study is in progress to find how the vibrios enter into the wall of the

intestines and whether they multiply in that site. (*iii*) The pathology of the alimentary tract is being investigated with particular reference to endothelial permeability leading to the huge loss of fluid and chloride.

2. The cytological study of cholera evacuation.

It is believed that the cholera evacuation is due to the aquaeous transudation from the lesions and that there is no inflammatory reaction. This cannot be confirmed both chemically as well as cytologically.

(*a*) Fifty cases have been selected and smears on slides made from samples of stools collected twice daily for 4 days or until death. These specimens show, in a good number of instances, the presence of a fair number of leucocytes often in clusters.

(*b*) The chemical examination of stool also points to the same thing. The protein content of 48 samples of stools taken from 20 cases daily for 3 days or until death is 1.8 grm. in the average with 4.7 grm. as maximum per 100 c.c. of the substance.

Further studies are in progress to determine the degree of inflammatory changes in the cholera intestine and its distribution in the alimentary tract.

3. Role of the presence of spirillum or spirochaetes in the alimentary tract.

Preliminary work has been done on the presence of spirillum or spirochaetes in the cholera evacuation and to evalute their presence with the severity of the clinical manifestations and also on the histological picture of the intestine. The study is awaiting further material during the next epidemic.

4. The result of the study of the pathology of pulmonary oedema in cholera has been published.

5. Study of bone marrow and suprarenal in cholera.

(*a*) A detailed study of bone marrow is in progress to determine the cause and nature of the haemopoietic response as leucocytosis is invariably present and that irrespective of the concentration of blood.

(*b*) A good collection of suprarenal glands from cholera post mortems has been made. The material is awaiting study.

6. Concentrated saline in the treatment of cholera.

The material so far obtained from the administration of concentrated saline in 6 cholera patients with collapse and high specific gravity of blood (varying from 1063 to 1066) and a few with pulmonary oedema shows that a quantity of 50 c.c. of a 20% solution of NaCl is quite sufficient to effectively lower the specific gravity of blood in a short time and serves the same purpose as that of 3 pints of hypertonic saline administered intravenously.

The idea of this form of treatment has been obtained from the writer's observation that hypochloraemia is a prominent finding in cholera and that it persists even after intravenous injection of saline, suggesting that in cholera there is more loss of salts than of fluids.

The effect after the administration of concentrated saline appears to be due to the drawing in of interstitial fluid into the circulation which exists in normal condition in a proportion of 3 to 1, i.e., interstitial fluid constituting

15% of the body weight while the blood fluid 5% of the body weight. This shows that even after collapse there exists a good amount of reserve in the interstitial fluid which can be drawn into the blood in order to lower the specific gravity. The only drawback is the intense headache appearing during the injection and lasting for sometime.

A few experiments have been performed. Twenty percent and 10% NaCl solution had been given in 20 guineapigs intraperitoneally, 4 guineapigs subcutaneously, 6 cats intravenously to determine the lethal dose and also effect on blood pressure, pulse, respiration, kidney function etc. Further animal experiments are in progress with or without glucose and other chemicals in the saline and also administration of atropin, adrenalin, pituitrin etc. to determine the pharmacological effect of concentrated saline before the method of treatment could be taken in hand during the next epidemic.

The result so far obtained is very encouraging. The growth quantity of 50 c.c. of 20% NaCl solution appears to serve the same purpose as an intravenous injection of three pints of hypertonic saline in the treatment of cholera.

MALARIA.

5. Malaria Institute of India (Research Section), Kasauli.

The research activities of the Institute have again been severely curtailed by the abnormal conditions brought about by the war. The post of Malaria Research Officer has remained unfilled, and four of the military assistant surgeons attached to the Institute have reverted to military duty. Three of these have been replaced by civil malaria assistants.

As in the previous year, a large proportion of the time of the staff has been taken up with courses of instruction in malariology. In addition to the annual six weeks' course for medical officers, three special courses have been held for personnel of the Defence Department, 122 of whom have received training at the Delhi Field Station since the outbreak of war. There was also a course for engineers similar to those held in 1940, which was attended by 26 engineers of various categories.

Publications.

Journal of the Malaria Institute of India—Two numbers containing 22 papers and 2 abstracts have been published during the year.

Scientific material sent to other workers.

A strain of monkey malaria has been supplied to the Director, Indian Institute for Medical Research, Calcutta. Collections of mounted specimens of mosquitoes and other biting insects of medical importance were sent to 16 different workers, including the Entomologist, Institute for Medical Research, Kuala Lumpur (F.M.S.) and O. C., Training Field Hygiene Section, Rawalpindi.

Testing of insecticides and sprayers.

About 150 experiments were carried out to test the mosquitocidal value of a number of insecticides, including pyrethrum flowers grown in Murree, Palampur and Kulu (Punjab) and in the Kurram Valley (N. W. F. P.). Flowers from all these sources gave very satisfactory results.

Further experiments were also carried out on aqueous insecticides in the concentrations recommended by the manufacturers. These were found to be somewhat inferior to the non-aqueous standard pyrethrum extract used for comparative purposes. Observations have also been made as to the effect of storage on the efficiency of certain insecticides previously tested.

A number of hand sprayers for use with pyrethrum insecticides have been tested. As a result of these experiments, the design of the hand sprayer evolved at this Institute has been further modified in the direction of simplicity. Over 1,000 sprayers have been individually tested for supply to the Defence Department.

Various types of knapsack oil sprayers have also been tested. As the result of a large number of experiments, a knapsack sprayer has now been designed which it is hoped will prove effective and economical in operation. All rubber diaphragms and washers have been eliminated, and the sprayer has been designed to withstand a fair amount of wear and tear under field conditions. A number of these sprayers have been tested for supply to the Defence Department.

In order to carry out comparative tests on the efficacy of various insecticides, including indigenous plants containing rotenone, on the body-louse, the breeding of these insects in the laboratory on a large scale has been started.

Testing of repellents.

Fourteen different types of pomades, both vanishing and greasy, containing different proportions of certain essential oils and pyrethrum extract, were tried to test their comparative repellent properties against mosquitoes. It was found that those containing not less than 7 per cent concentrated pyrethrum extract (containing 2 per cent pyrethrins) gave the best results.

Entomological researches.

The colony of *A. stephensi* (type form) started in 1939 has been successfully maintained and has now reached its 39th generation without apparent ill-effects to the individual insects. Observations regarding the effect of cold weather on the metabolic activities of these insects have been continued.

The colony of *Aedes aegypti* (Karachi strain) has been maintained as heretofore. Another colony of the local (Delhi) strain has also been started. Though individuals of this species from these two places appear to be morphologically identical, they show certain biological differences.

One hundred and thirty-two collections of mosquitoes have been received for identification from various parts of India during the year. Collections of mosquitoes from the Middle East and West Africa have also been received from Professor P. A. Buxton, London School of Tropical Medicine, and from North Borneo from Dr. McArthur.

Field researches.

(*a*) *Delhi field station*—Epidemiological studies have been carried out in a number of villages including periodical spleen and blood examinations and dissections of mosquitoes, with special reference to the spray-killing of adult mosquitoes with pyrethrum insecticide. The antimalaria operations in Delhi have been directed and supervised as in previous years, and a number of investigations directed to the improvement of control measures have been undertaken. Special studies have been carried out with reference to the prevention of larval drift in irrigation canals, together with comparative studies to test the value of certain oils and the methods of applying these to breeding places. The employment of *Gambusia* fish in ornamental waters has been extended.

(*b*) *Orissa field unit*—Epidemiological observations have been carried out in a number of localities on the shore of the Chilka Lake and in the area immediately to the south of it. The incrimination of *A. sundaicus* as the sole cause of malaria incidence in this area has been confirmed. Special attention has been paid to the testing of methods for controlling the breeding of this species. A large amount of data has been collected regarding the bionomics of the local anophelines and the association of their larvae with various types of aquatic vegetation.

(*c*) *Kurram Valley* (*N.W.F.P.*)—Investigations have indicated that *A. stephensi* is the principal carrier in the early part of summer, and *A. culicifacies* in the autumn. Recommendations have been made for the control of malaria in this area.

(*d*) *Ahmedabad field unit*—A field unit has been investigating malaria conditions in this city for a period of six months from 12.5.41 with the object of formulating a programme for the control of malaria and of the local mosquito nuisance. The results of mosquito dissections indicate that *A. stephensi* is the principal local malaria vector.

(*e*) *Daurala (near Meerut)*—A field unit investigated malaria conditions in the vicinity of Daurala Sugar Works during July 8-13 and September 22-27.

LABORATORY RESEARCHES.

(1) A number of monkey malaria strains have been maintained in the laboratories. (2) Observations on the behaviour of monkey malaria strains have been continued with special reference to the appearance of some unusual forms (band forms) in *P. knowlesi*. It has been observed that these atypical forms appear when the infection is heavy in untreated cases, whereas in low-grade chronic infections, such forms are seldom met with. (3) The preparation of high titre anti-man, anti-cow and anti-horse sera for use in the precipitin tests for determining the source of blood meal of mosquitoes has been continued. It has been found that in anti-man serum prepared from white rabbits the titres fall rapidly on storage. Experiments are in progress for the preparation of anti-man serum from fowls. (4) Attempts have been made to secure the transmission of monkey malaria (*P. knowlesi*) by *A. subpictus*, *A. stephensi*, *A. annularis* and *A. culicifacies*, but the results have not been successful, except for one gut infection in *A. culicifacies*. (5) A sample of Leishman stain powder prepared in India was tested but found to be less satisfactory than the original preparation. (6) A sample of Wright's stain powder was prepared in these laboratories with eosin made in India, and was found to be suitable for the routine examination of blood films for malaria parasites.

6. Malaria transmission enquiry under Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta.

Practical work on the study of temperature and humidity as limiting factors in malaria transmission by *A. culicifacies* was continued till the end of March, when the enquiry was closed except for the preparation of the detailed report. In the time available, an effort was made to complete the data which would give some idea of the effect of temperature at least. Intensive investigation at each combination of temperature and humidity was therefore not possible. In all, about 450 *A. culicifacies* were dissected after feeding on gametocyte cases. Excluding mosquitoes in entirely negative lots, (due chiefly, as has already been reported last year, to the comparatively low degree of susceptibility of this species), the accompanying table summarises the results of exposing mosquitoes to different temperatures, the relative humidity being moderately high (70-90 per cent) :—

Table.

Temperature °F	Percentage total infection.			Percentage total infection of control lots at 80°F.		
	P. vivax	*P. falciparum*	*P. malariae*	*P. vivax*	*P. falciparum*	*P. malariae*
94	0/19 = 0	0/28 = 0	..	6/15 = 40	8/16 = 50	..
90	3/11 = 27.3	1/13 = 7.6	..	3/6 = 50	0/7 = 0	..
59.6[1]	1[2]/7 = 14.2	1[2]/19 = 5.2	0[3]/9 = 0	1/2 = 50	6/18 = 33.3	4/15 = 26.6

1. Mean temperature, based on readings for 2-hour periods, of variation between 67° and 54°F; for seventy per cent of the total time occupied by these experiments the temperature varied between 62° and 57°F.
2. Only oocysts observed.
3. Owing to the long incubation of *P. malariae* the mosquitoes were transferred after 9 days at these conditions to 80°F to hasten development of the parasites, if viable, and were examined after appropriate intervals.

Although the number of observations in each group is smaller than was desired, the results indicate that when the relative humidity is between 70 and 90 per cent, generally considered to be a very favourable range for transmission, the development of *P. vivax* and *P. falciparum* is prevented when the temperature is continuously a few degrees above 90°F. This was confirmed for *P. falciparum* by subjecting oocysts partially developed under favourable conditions to a temperature of 94°, when degeneration occurred, frequently resulting in the production of " black spores ".

From the low infectivity rates obtained under cold conditions, represented by a mean temperature of roughly 60°F, this latter figure, would appear to be outside the range for effective transmission of *P. vivax* and *P. falciparum*, if not the actual lower limiting value. Although only oocysts were observed, it is believed that the sporogony could be completed, though requiring at least twenty days, because oocysts (*P. falciparum*) brought to an advanced state of development under more favourable conditions, matured when kept at the low temperature range.

The upper limiting value of temperature for transmission of *P. malariae* has not been ascertained. A mean temperature of about 60°F is apparently below the lower limiting value, since the parasite failed to reach even the oocyst stage when brought to favourable conditions after a limited period of exposure to cold. In another lot at 80°F, 100 per cent gland infection was obtained. The general success achieved in infecting *A. culicifacies* with *P. malariae*, which is admitted by all workers to be the most difficult of the three common parasites of man with which to infect mosquitoes, leads one to suggest that this mosquito may, in some way, be peculiarly susceptible to the quartan parasite.

With regard to the role of humidity, it can be stated that a relative humidity of 50 per cent is by no means inimical to the development of *P. vivax*, provided the temperature is such that the loss of water is not so rapid as to kill the mosquitoes. For a good gland infection was observed after eight days in one *A. culicifacies* surviving out of three fed and exposed to this humidity, the dry bulb temperature varying between 75° and 80°. Above 94°, with the same relative humidity, the mosquitoes, did not survive more than three days, a period almost certainly too short to allow development of any malarial parasite.

The detailed final report of the work done with *A. culicifacies* is nearing completion.

7. Enquiry on a species of avian malaria under Dr. B. C. Basu at the Imperial Veterinary Research Institute, Mukteswar.

Early in this year the writer had to undertake the preparation of the Malaria Transmission Memoir which has now been completed and sent to Col. Covell. It is presented in two sections ; (*a*) a review of the whole of the previous literature on the subject (1901-1940) ; and (*b*) a presentation of their own results of work for eight years. In all 186 papers and reports in various languages have been studied. The Memoir contains 160 typed pages and includes a large number of tables, charts, graphs and text-figures. He was occupied with this work for several months and has now been engaged for sometime past in preparing two more reports on malaria transmission work. All these works were conducted with a grant from the Indian Research Fund Association. Abstracts of two papers, namely, (*i*) atmospheric temperature and humidity in relation to experimental transmission of malaria with *Anopheles annularis ;* and (*ii*) a survey of the Anopheline fauna and malaria at Izatnagar, have been sent for the coming Indian Science Congress to be held at Baroda in January 1942.

Through the courtesy of Col. Shortt, a strain of *P. gallinaceum* has been received and is being maintained in this laboratory. The course of the disease has been studied in the fowls and the nature of the parasite in vertebrate and invertebrate hosts is under investigation. In the series of experiments, birds recovered from this infection, have been observed to develop immunity against further infection with this parasite (this subject is being further studied). The Director of the Mukteswar Institute has kindly provided all possible facilities for the prosecution of this work.

In last year's note on the work done on the Avian Malaria Enquiry, the progress and scheme of the work were given. The internal organs particularly the capillaries of the brain of a number of pond herons infected with *Plasmodium heroni*, were examined for evidence of exo-erythrocytic schizogony but in none, so far, has this been found. Blood films taken at various intervals, are under study to ascertain the duration of schizogony of the parasite.

It is proposed to continue and complete the following series of observations :—

(*a*) To study the exo-erythrocytic schizogony of *P. heroni* particularly in the capillaries of the brain.

(*b*) To determine the duration of the schizogony cycle of *P. heroni* in the paddy birds.

(*c*) To work out the complete sporogony cycle of *P. heroni* in the mosquitoes.

8. Enquiry into the life history of the weeds in the Chilka Lake under Mr. P. Parija, Principal, Ravenshaw College, Cuttack.

The report relates to the work done during the calendar year of 1941.

DISTRIBUTION OF THE WEEDS.

Weeds, both phanerogamic or cryptogamic, show a zonal distribution and they grow near the shores of the mainland or the islands. In some cases the distribution extends over a mile from the shore while in other cases it is only a few yards from the beach. This zonal distribution is probably due to the difference in soil conditions, salinity and depth.

The soils in different parts of the lake shore were analysed to determine the relation between weed growth and the nature of the soil. It was found that some weeds like *Potamogeton*, *Ruppia* and *Halophila stipulacea* prefer richer soil than others.

GROWTH OF WEEDS.

The list of weeds given below indicates the period during which they grow in the lake :—

A. *Weeds growing throughout the year*

(*Phaneragamic*)		
Halophila ovata		
H. Stipulacea		
Naias	—	*very* poor growth in hot season.
(*Cryptogamic*)		
Gracilaria lichenoides		abundance and formation of cystocarps in dry months.
G. confervoides		
Ceramium	}	abundance in winter.
Polysiphoria	}	
Diatoms		
Enteromorpher	—	poor growth in April and May.
Chaetomorpha	—	very poor growth in dry months.

B. *Weeds growing in different seasons.*

(*Phanerogamic*)	
Potamogeton pectinatus	abundance from October to March ; perish in dry season when salinity reaches its maximum.
Ruppia rostellata	Growing season from August to March. Maximum growth in January and February.
(*Cryptogamic*)	
Lyngbya	August to March.
Grateloupia	December to June.

Cladophora	July to March.
Stigeoclonium	November to February.
Oedogonium	September to January.
Phormidium	August till the end of February.
Stirogyra, *Anabaena*, *Nostoc*	in the rainy season.

Potamogeton pectinatus is the most dominant flowering weed. It grows mainly in two seasons of the year, one in the beginning of the rains and the other in the beginning of the cold weather. This biseasonal growth is conditioned by the salinity of the lake water which varies from season to season. The weed growth has an optimum salinity and this occurs in the two seasons mentioned above. The plant propagates both vegetatively and sexually, the former by means of tubers, winter buds and creeping root stocks and the latter by means of seeds.

The dormancy of the seeds has been investigated and it is found that it extends over a year. *Lyngbya* with three species is the most dominant cryptogamic weed and is distributed all over the lake. In winter it grows under water in thick mats and when the old filaments begin to decay they float up in masses and become a nuisance.

Chemical analysis of water.

Salinity—Salinity of the lake water having some relation with weed growth, records of salinity at various stations were kept throughout the year. During the rains, the water of the northern portion of the lake becomes almost fresh while that of the southern portion maintains a high salinity throughout the year. The following table gives the chlorine content from the north to the south at a few stations on the lake throughout the year.

Table I.

Chlorine content in gms. per 100,000 *c.c. of water at three places given in order from north to south.*

Locality	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Kaluparaghat	205.5	353.75	462.5	462.5	617	897.5	223.75	245	77. 5	36.5	93.75	..
Balugaon ..	280.5	327.55	397.65	503.75	992. 5	1493.95	1337.5	460	372. 5	257.5	442.5	581.25
Rambha ..	362.5	422.5	462.5	511.3	633.75	1752.5	1345	1311.75	1095	755	803.75	..
Parikud ..	3.6315	4.025	4.575	6.2137	6.5375	16.0112	12.5625	7.7575	6.65	6.4875	5.625	..

Hydrogen-ion-contentration—In order to find out whether hydrogen-ion-concentration has any relation to the weed growth, flowering or algal, records were kept of the hydrogen-ion-concentration of the lake water at various places throughout the year. The hydrogen-ion-concentration is higher near the shore than in the interior. The pH does not seem to have any relation to weed growth.

Oxygen—During the rains many of the flowering weeds grow under water at a considerable depth. In some cases up to 10 or 12 feet. If they do not seem to suffer much in this condition, it was desirable to measure the oxygen concentration of the lake water during the rains. The oxygen concentration varies from place to place and from depth to depth at a particular place. It is greater in muddy water than in the fresh. The bearing of this will be discussed in the final report.

Light.

The water of the northern portion of the lake becomes very turbid during the floods and yet submerged plants continue to grow. Again when the water becomes clear, some weeds thrive under water at a depth of 7 or 8 feet. As these weeds must be carrying on Photosynthesis, the question of light penetration into water, both muddy and clear, became important. Penetrability of the light into the lake water at different places was measured throughout the year. Two methods were adopted, one, by noting the visibility of a white disk lowered into the lake water and the second, photographic method. The bearing of these records will be discussed in the final report.

Depth, density and other records.

Collection of data regarding depth and density has been made all over the lake month by month. In summer the depth rarely extends 8 feet in the southern portion of the lake while in the remaining part of the lake it hardly reaches 6 feet. The maximum depth recorded is 14 feet near Kalijai island during the rains.

Density varies naturally from month to month. Metereological data have been collected at various stations.

Chemical analysis of the weeds.

With a view to finding some use for the weeds, the dried weeds have been analysed. The results of the analysis are given below:—

Table II showing the result of analysis of the weeds.

Samples	Moisture	Organic matter	Ash	Total N_2.
Potamogeton ..	15.89	65.5	18.52	1.443
Naias ..	14.06	56.12	29.82	1.588
Ruppia ..	15.88	63.8	22.32	1.696

Table III showing the result of analysis of ash.

Samples	Potash K_2O	Phosphate P_2O_5	Lime CaO	Iodine
Potamogeton ..	17.06	3.203	3.48	+++
Naias ..	11.12	0.9513	2.388	++++
Gracilaria ..	7.11	0.817	3.22	++
Ruppia ..	19.99	3.3382	0.507	+

Table IV showing the % of iodine in kelp.

Chilka kelp		Kelp commercial
Potamogeton ..	0.0026	
Naias ..	0.004	0.5 to 1.3
Gracilaria ..	0.0028	c.f. In. Ch. Mellor. Vol. II p. 42.
Ruppia ..	0.0043	
Grateloupia ..	0.03	

Although the iodine content is not so large as in commercial kelps, the possibility of extraction of iodine from the weeds deserves exploration by those interested.

OBSERVATION AS TO THE FLOATING OF WEED MASSES.

Observations were taken in various seasons to find out the reasons why the weeds break up and float in masses. The data collected under the various headings noted above have all a bearing on the floating of the weeds. In addition to these, certain animals such as the bivalves, birds, fish and other lake animals play a part in this matter. Last, though not the least, fisherman's net also is responsible to some extent.

9. Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

Investigations during the year 1941 were concerned with the pharmacological action of sulphathiazole with special reference to its effect in wounds and burns and the problem of immunity in monkey malaria.

I. SULPHATHIAZOLE.

(*a*) *Pharmacological action of sulphathiazole*—Some pharmacological actions of this new important sulphanilamide derivative were investigated during the year under report. The drug was found to exert very little action on the cardio-vascular, gastro-intestinal, genito-urinary and central nervous systems in acute experiments when given intravenously in doses much larger than those used in therapeutics. When the soluble salt was applied directly to the central nervous system by a ventricular or cisternal puncture however a stimulant action was observed.

The rate of absorption was found to be very rapid by the oral route of administration. The drug was quickly absorbed and rapidly excreted. The channel of excretion appears to be confined to the kidneys. The drug is acetylated in the body and the liver seems to be the organ chiefly responsible for this acetylation. The degree of acetylation in different species of animals varied. When excretion of the drug through the kidneys was prevented, the major portion of the drug in the blood was found to be in the acetylated form.

The resistance of the placental barrier to allow the drug to permeate through into the faetal circulation was investigated and it was found that the drug could permeate through fairly easily. Whether this permeability affects the faetus adversely is being investigated. Similarly the appearance of the drug in the cerebro-spinal fluid is being investigated and preliminary experiments show that the drug can be found in measurable amounts in the cerebro-spinal fluid, the degree of concentration depending upon the amount of drug in circulation.

(*b*) *Action of sulphathiazole in wounds*—Local packing with sulphanilamide derivatives has been advocated in recent times for treating crushed injuries. This problem was investigated in experimental animals in whom artificial injuries were inflicted under anaesthesia and the wounds contaminated with strepto and staphylococci. Different sulphanilamide derivatives were then packed into the wound and it was found that sulphathiazole gave the best results. These preliminary investigations are now being carried out on a wider scale.

(*c*) *Action of sulphathiazole in burns*—The question of local treatment of burns has attracted considerable attention in the medical world at present and the advantages and disadvantages of various forms of treatment are being discussed. A few methods at present employed were tried experimentally and it was found that a suspension of sulphathiazole in cod-liver oil gave very promising results. These investigations also are being continued on a wider scale.

II. Immunity in monkey malaria.

As reported last year the question of 'humoral' and 'cellular' factors in malarial immunity in the rhesus monkey was investigated by the cross circulation technique, heparin being used as an anti-coagulant. The stock of heparin being exhausted, an alternative method of cross circulation by the citrate method was evolved. Preliminary experiments were made to determine the amount of citrate needed for each experiment and to see whether the quantity of citrate used, induction and maintenance of anaesthesia and the surgical interference involved influenced the degree of immunity or viability of the parasites in any way. Replacement of blood of monkeys was then effected by this new method and the results studied on a quantitative basis. These results may be summarized as follows:—

(*i*) Even when nearly 80 per cent of the blood of an immune monkey is removed and replaced by non-immune blood obtained from normal monkeys, the immune monkey still retains its immunity to *P. Knowelsi* infection.

(*ii*) When 75 per cent or more normal blood from a normal monkey is removed and replaced by immune blood obtained from immune monkeys the normal monkey acquires immunity. If the replacement is 50 per cent the normal monkey may or may not acquire immunity and when it is 33 per cent or less, the normal animal fails to acquire immunity. In experiments

with only 50 per cent replacement of normal blood with immune blood, results were obtained which suggest that a time factor determines the final outcome. That is when two weeks or more elapse between replacement and infection, the monkey fails to get the infection but if the interval is less than one week, it succumbs to the infection.

(*iii*) If 70 per cent or more of infected blood from a freshly infected monkey (with a parasite count of about 1000 per 10,000 R.B.Cs.) is removed and replaced by immune blood obtained from immune monkeys the infected monkey gets rid of the infection and passes into a chronic infection stage.

These experiments therefore show that both the 'Cellular' and 'Humoral' agencies play their part in conferring immunity in malarial infections. These studies are still being pursued.

NUTRITION.

10. Nutrition researches under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, Coonoor.

The work carried out during the year included the following:

The composition of foodstuffs.

During recent years special attention has been given by the Laboratories to the development of satisfactory chemical tests for various members of the B group of vitamins, which is of great clinical and public health importance in India. Biological tests for vitamins are in general laborious and time-consuming, and have a high margin of error. Chemical tests can be rapidly carried out and provide a valuable weapon of research.

A satisfactory fluorometric method of testing for riboflavin has been worked out. The various physico-chemical methods hitherto reported in the literature are in general limited in value because, when applied to certain food products, they are either not specific for riboflavin or else all the riboflavin present in a food is not estimated. The chief sources of error are: incomplete extraction of riboflavin; losses occurring during the various processes of purification; interference caused by the presence of varying amounts of coloured and fluorescent impurities in the extracts employed for estimation.

These difficulties appear to have been satisfactorily circumvented and the method evolved can be readily applied to any kind of food or biological substance. Values obtained for various common foods were as follows:

Riboflavin content of certain foodstuffs.

Name of foodstuff	*Botanical name*	Riboflavin µg./g.
Rice, raw, milled		0.3
Rice, parboiled, milled		0.8
Rice, polishings, raw		3.0
Ragi (*Eleusine coracana*)		0.5
Whole wheat		1.2
Red gram (*Cajanus indicus*)		2.5
Black gram (*Phaseolus mungo*)		1.8
Black pepper (*Piper nigrum*)		0.7
Cabbage (*Brassica oleracea capitata*)		0.5
Carrot (*Daucus carota*)		0.3
Orange (*Citrus aurantium*)		0.4
Papayya (*Carica papaya*)		0.3
Tree tomato (*Cyphomandra betacea*)		0.4
Yeast, dried, brewer's		55.5
Whole milk		1.5 µg./c.c.

The foods which bulk largely in ordinary Indian diets are in general not good sources of riboflavin, and it has been found that deficiency disease associated with lack of this vitamin is widespread.

The test for vitamin B_6 (pyridoxin), devised in the Laboratories, was employed in the further study of the pyridoxin content of foods. Improvements were introduced into the cyanogen-bromide test for nicotinic acid, also originally evolved in the Laboratories. Finally, the thiochrome method of assaying vitamin B_1 has been adapted to make it suitable for application to any type of foodstuff. These various chemical tests for the B vitamins provide a weapon of research of which abundant use can be made.

The vitamin B_1 and nicotinic acid content of different grades of wheat flour commonly consumed in India was investigated. It was found that most varieties of " atta " in common use are good sources of both these food factors.

Numerous samples of fish liver oil have been tested for vitamin A, and the vitamin D content of fish liver oils has been further studied. The vitamin A content of shark liver oil has been found to be very variable. The richest sample assayed during the year gave the remarkably high value of 190,000 international vitamin A units per gramme, but in general values above 20,000 were given only exceptionally by the samples investigated in the Laboratories. The physical and chemical constants of shark and saw-fish liver oils have been studied. In view of the present importance of cod liver oil substitutes in India, a re-examination of physical and chemical methods for estimating vitamin A in fish liver oil was undertaken. Spectrophotometric and tintometric methods of estimation were applied to 24 samples of shark and saw-fish liver oil and the ratio $\frac{\text{international units}}{\text{blue units}}$ was found to be on the average 53. The suggestion has been put forward that if it is desired to express blue values obtained on shark liver oil in terms of international units, a conversion factor of 50 to 55 may justifiably be used, provided the blue values are obtained on the non-saponifiable fraction.

Considerable progress was made during the year in the production of cod liver oil substitutes, based mainly on shark liver oil, at various coastal centres. These substitutes are now available in fairly large quantities and are being widely used in India. This promising industry was initiated by the Laboratories and its development has been furthered by supplying advice and carrying out tests.

The routine investigation of the chemical composition of foods not hitherto analysed has been continued. The new edition of Health Bulletin No. 23 : " The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets ", contains analyses of 300 foods, as compared with 200 in the first edition.

Various animal experiments.

The metabolism of vitamin B_6 in rats was studied, the method of estimating vitamin B_6 developed in the Laboratories being adapted for the determination of the vitamin in urine. In rats fed on a diet deficient in vitamin B_6, excretion was in excess of intake. Rats fed on the same diet supplemented by 10 microgrammes of vitamin B_6 daily excreted about 40 per cent of intake. Various tissues of the rats fed on the deficient diets contained less vitamin B_6 than similar tissues of rats in the other group. Somewhat similar results were obtained in experiments of the same nature with riboflavin. Rats receiving additional riboflavin excreted about 45 per cent of intake. The riboflavin content of the liver, skeletal muscles and heart muscle of rats fed on diets deficient in riboflavin was lower than that of the same tissue in animals receiving additional riboflavin and animals fed on the stock diet.

Attempts to develop macrocytic anaemia in monkeys by feeding them for long periods on deficient rice diets failed.

Numerous biological tests to determine the nutritive value of different foods were carried out during the year. Demonstration animal experiments were staged for the benefit of the nutrition class.

Pathology.

Monkeys fed on deficient rice diets tend to develop fatal diarrhoea. In such animals atrophic changes in the small intestine, most marked in the ileum, have been observed. Recently a worker in East Africa* has described a condition in human beings resulting from dietary deficiency, to which the name "nutritional diarrhoea" was given, which appears to be identical, as regards pathological changes, with that observed in the monkeys. It thus appears that this human disease has been experimentally reproduced for the first time. The large amount of pathological material obtained from monkeys in these investigations has now been analysed and is ready for publication. It is hoped to extend this work by the pahtological study of human material. At its present stage the investigation suggests that the nutritional element is of importance in connection with the high mortality from "dysentery and diarrhoea" in India.

"Tropical ulcer" was found to be very common in malnourished labourers in Coorg. A pathological study of this condition has not revealed any new features not already reported in the literature.

Miscellaneous investigations.

The excretion of vitamin B_6 by human beings was studied. It was found that the ingestion of pure vitamin B_6 caused an increase in excretion during the first 3 hours, but that only 5 per cent of a test dose of 50 milligrammes was excreted. The average daily excretion of subjects on an oridnary rice diet was found to be about 500 microgrammes.

In the case of riboflavin, the percentage excretion by a normal subject of doses ranging from 1 to 10 mg. was much higher—from 80 to 85 per cent. On the other hand, in patients suffering from riboflavin deficiency, the administration of pure riboflavin scarcely increased excretion, suggesting that the additional vitamin, required by the tissues, was rapidly utilised. These observations provide a basis for tests to determine mild degrees of riboflavin deficiency in human beings.

A concentrate of vitamin A for parenteral injection was prepared from shark oil. The potency of this material compared well with that of various proprietary preparations now on the market. Some of the concentrate was given to Lieut.-Colonel E. W. O'G. Kirwan, C.I.E., I.M.S., The Eye Infirmary, Medical College Hospitals, Calcutta, for trial in cases suffering from ophthalmic signs and symptoms of vitamin A deficiency. Excellent results are reported. The possibility of developing the production of such preparations from shark liver oil in India has thus been demonstrated.

---

* McKenzie. East African Medical Journal, 1940, 17, p.30.

Field work.

In all Eastern countries apart from India in which beriberi is prevalent, the disease is known to be common among infants. It usually attacks breast-fed infants at about the third month of life, and is often fatal. Infantile beriberi has not, however, been reported in India. During visits to the Northern Circars the opportunity arose of observing clinically infantile cases which were unquestionably of this nature and this gave rise to the suspicion that infantile beriberi, though generally unrecognised, may be a serious problem in this area. Epidemiological investigations in hospitals and out-patient departments present considerable difficulties and the problem was approached by a study of the existing vital statistics relating to infantile mortality in a number of towns in the beriberi area and in other parts of the country.

The Annual Reports of the Director of Public Health, Madras, provide data about infantile mortality in municipalities in the Madras Presidency. Infantile deaths are grouped under the periods 0-1 month, 1-6 months, and 6 months to 1 year. A comparison was drawn between the proportionate mortality in these periods in 17 towns in the Northern Circars and 17 towns in the province south of Madras city in 1938, the total population concerned being approximately the same in each case. Only the records of municipalities employing health officers were investigated, because in such towns registration of births and deaths is likely to be fairly accurate. The results were as follows:

Percentage of total infantile mortality.

	0–1 months	1–6 months	6–12 months
Towns in beriberi area (raw milled rice) ..	35.0	41.3	23.7
Towns outside beriberi area (parboiled milled rice) ..	53.0	26.6	20.4
British India (Report of the Public Health Commissioner with the Govt. of India, 1938) ..	46.8	30.6	22.6

In the beriberi area infant mortality reaches its highest peak during the period 1-6 months. In the other towns and in British India generally, the greatest proportionate mortality occurs during the first month. Further analysis showed that the reported infantile mortality in towns in the beriberi area is considerably higher than in towns outside the area, and that the excess mortality in the former is due to the larger number of deaths occurring in group 1-6 months.

Municipal records were scrutinised to elicit the proportion of deaths at each month of infancy in three of the largest towns in each group, over a period of 10 years. In the towns in the beriberi area there was a striking peak in mortality at the third month, which was constantly present in all years and throughout the records of the 3 municipalities in question. It is highly probable that this peak, which was entirely absent in the records of the municipalities outside the beriberi area, is due to deaths from infantile beriberi.

The inaccuracy of vital statistics in India is well known. Registration of births and deaths in the Madras Presidency is, however, more complete than anywhere else in India and urban areas were concerned in which whole-time

health officials were employed. Further, it is difficult to imagine how a sharp and constant peak in mortality at the age of 3 months, which revealed itself only on detailed study of the records, could be produced by error and omissions in registration.

Superficial keratitis due to riboflavin deficiency has been described in India for the first time. Numerous cases were observed in the Government Ophthalmic Hospital, Madras. Typical cases show superficial opacities in the cornea and circumcorneal injection, and complain of a burning sensation in the eyes, photophobia, lacrymation and dimness of vision. The ophthalmic condition is usually accompanied by angular stomatitis and fissuring of the tongue. In males a rough scaly condition of the skin of the scrotum is often observed. The effect of injections of pure riboflavin on the eye lesions is immediate and striking, improvement being often observed within 48 hours or less. A suitable initial dose is 4 milligrammes of riboflavin. Oral administration is less immediately effective than parenteral injection. While relief on treatment with the pure vitamin is usually rapid, there is a tendency towards recurrence when treatment is stopped and the patient returns to his customary riboflavin-deficient diet. It is not easy to suggest to poor patients a suitable diet rich in riboflavin. Milk is a relatively good source, but many patients cannot afford to take milk in sufficient quantities. Liver is rich in the vitamin, but not a customary food in India. Dried yeast is also a rich source, but yeast is at present expensive and difficult to procure. Further study of the riboflavin content of Indian foods may solve the difficulty of devising a diet which can be recommended to poor patients to prevent the recurrence of keratitis after treatment, and generally advocated for prevention.

Haematological investigations were carried out in Coorg Province. Malaria and hookworm are prevalent in this area and surveys have revealed that the diets commonly consumed are deficient in many respects and that malnutrition is widespread. A severe degree of anaemia in pregnant women is frequently observed. Determination of the haemoglobin content of 150 pregnant and 38 non-pregnant women gave the following results:

		Hb grammes per cent	Standard deviation	Co-efficient of variation
Pregnant women ..	150	8.16	±2.58	±31.6
Non-pregnant women ..	38	10.13	±0.93	± 9.13

Of the 150 pregnant cases 52, or 35.3 per cent, gave a haemoglobin figure below 7·7 grammes per cent. In 15 per cent the Hb content of the blood was below 5 grammes per cent. The problem of anaemia in Coorg appears to be very similar to that in Assam.

An experiment was carried out in which cheap tablets containing ferrous sulphate were distributed regularly to school children. The effect of this supplement on Hb levels was observed, untreated children serving as controls. A statistically significant rise in haemoglobin was recorded.

Dental examinations of 6,866 children in various parts of India have been carried out within recent years, to determine the incidence of caries. The percentage of all children free from caries was 44·5, a percentage which may be compared with those recorded in London and Sheffield, 1·9 and 2·5 per cent

respectively. Urban children in all age groups showed more caries than rural children. Wheat-eaters had slightly less caries than rice-eaters. The highest incidence of caries in permanent teeth observed anywhere in India was found in an Anglo-Indian orphanage, where the diet was in many respects superior to that consumed by Indian children in general, but included bread made from refined wheat flour and fairly large quantities of sugar. This is a finding of interest. On the whole dental caries does not appear to be a problem of major public health importance in India. Since the standards of health and nutrition of children in Indian schools are in general inferior to those of European and American children, it is of interest to note that they have the advantage at this particular point.

Investigations on yeast.

The growth of various strains of yeast in solutions of molasses has been studied. It has been observed that molasses-yeast is usually poorer than brewer's yeast in vitamin B_1, but is rich in riboflavin, nicotinic acid and vitamin B_6. An abundant cheap supply of molasses-yeast in India would be of great value, particularly in the treatment and prevention of riboflavin deficiency. Pure riboflavin is an expensive product. Certain strains of yeast when grown on molasses are, in the dried form, much more palatable than dried brewer's yeast.

Training course for health officers.

The usual course was given during the months April to June. The class included 26 members, from many different parts of India. This was the fifth annual class, and the course now follows well-established lines. Contact was maintained between the Laboratories and nutrition officers and others in various Provinces and States who have taken the course in previous years.

Education and propaganda.

A third edition of Health Bulletin No. 23 was prepared during the year. A new Health Bulletin, "Food and Diet", for use in schools, has also been written. Additions have been made to the museum, which now includes numerous pictures, charts and exhibits. Enquiries embracing many aspects of nutrition continue to be received from all over India.

11. Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

Role of ascorbic acid in infection.

Previously it has been reported that patients suffering from acute pulmonary tuberculosis excrete relatively more combined ascorbic acid and less free ascorbic acid in the urine than normal subjects. Administration of a single high dose of ascorbic acid (700 mg.) did not result in an increased elimination of combined ascorbic acid. The prolonged administration for a peiod of

12 to 15 days of 700 mg. of ascorbic acid daily resulted in a drop in the excretion of combined ascorbic acid. In normal subjects the disappearance of combined ascorbic acid from the urine was quicker on the administration of large doses of ascorbic acid. Similar results have been obtained with the guinea-pigs injected with 100 mg. of ascorbic acid.

Vitamin C and micro-organisms.

It has been demonstrated that vitamin C in a concentration of 1/20,000-1/15,000 has an inhibitory effect on the growth of the following bacteria—*B. typhosus, B. subtilis, B. coli, Aerobacter, aerogenes, Staphylococcus aureus, Streptococcus haemolyticus and C. diphtheria* in artificial media. Vitamin C, however, exerts a stimulating action in 1/50,000 concentration and an inhibitory action in 1/10,000 concentration on the growth of certain fungi in artificial media.

The effect of certain drugs and poisons on the excretion of ascorbic acid by the rat and the guinea-pig.

In continuation of the researches on the possible detoxicating function of ascorbic acid in the guinea-pig, the effect of administration of different toxic substances like histamine, phenol, camphor, chloral hydrate, copper, phosphorus, fluoride and cyanide, has been carried out. Histamine, phenol and camphor led to increased elimination of combined ascorbic acid, as was previously observed after injection of bacterial toxins like those of diphtheria and tetanus. The other substances did not cause a rise in the output of combined ascorbic acid in the urine. A detailed investigation has been published.

It has further been observed that narcotics like chloretone, paraldehyde, phenobarbital, urethane, amidopyrine and bromobenzene when fed to the rat, caused increased excretion of free ascorbic acid with complete disappearance of combined ascorbic acid (Cf. Longenecker, Fricke and King, *J. Biol. Chem.*, 1940, **135**, 497). These same narcotics produced also an increase in the urinary excretion of free ascorbic acid in the guinea-pig though to a much less extent than in the rat. Striking differences between the narcotised rat and guinea-pig were, however, observed on examination of the ascorbic acid content of the tissues of the two species. A depletion of tissue—ascorbic acid was observed in the case of the guinea-pig which was comparable with the effect of chloral hydrate, camphor, etc. and diphtheria and tetanus toxins, previously reported. On the other hand the ascorbic acid content of the rat tissues was increased. It would appear therefore that the effect of the narcotics on these two species of animals is different, which may really be due to the fundamental difference that the rat can normally synthesise vitamin C, while the guinea-pig cannot. The increase in tissue ascorbic acid of the rat is particularly noticeable in the kidney and liver.

Ascorbic acid, ascorbigen and haemoglobin in blood during normal and scorbutic conditions.

The presence of bound ascorbic acid in many plant foodstuffs as well as in different animal tissues, has been established. Investigations on the existence of ascorbigen in the blood of normal human subjects, rabbits, guinea-pigs, rats,

fowls and pigeons have been carried out along with the estimation of free ascorbic acid and haemoglobin content. It has been observed in experiments with the guinea-pig that there is a diminution in the concentration of all these substances in blood in scurvy. Administration of ascorbic acid restored the haemoglobin level. This restoration of haemoglobin level was found by controlled experiments with guinea-pigs with restricted food-intake to be not simply due to the consumption of more diet and therefore of more iron on the administration of vitamin C. Some direct relation between ascorbic acid and the formation of haemoglobin seems to exist. With the progress of scurvy blood ascorbigen suffers a more rapid diminution than free ascorbic acid in blood and with the progressive cure of scurvy by administration of ascorbic acid, the ascorbigen level undergoes a more rapid restoration than the ascorbic acid level. The function of ascorbigen in blood thus seems to be a sort of store house of ascorbic acid from which free ascorbic acid can be released for direct utilisation when the body needs it.

A COMPARATIVE STUDY OF THE CHEMICAL AND BIOLOGICAL METHODS OF THE ESTIMATION OF TOTAL ASCORBIC ACID IN CABBAGE JUICE.

The method of assay of ascorbic acid by the use of 2 : 6-dichlorophenol-indophenol has been long established, but it is still sometimes disputed how far the method gives specifically the value of the "true, total" ascorbic acid. A modified method involving the use of ascorbic acid oxidase by which the specificity of the reaction is increased and by which the total value comprising the free ascorbic acid, ascorbigen and dehydro-ascorbic acid is obtained, has been developed. It was, however, important to know how far this modified chemical method is in agreement with the results of biological assay. This investigation has now been carried out with cabbage juice and it has been found in experiments with guinea-pigs by the use of the tooth method that the results of biological assay are most in agreement with the results of the modified method, previously reported. The classical method involving simple titration with the indophenol indicator seems to give somewhat lower values.

12. Nutrition investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

PROTEIN, FAT AND MINERAL CONTENT OF FISH.

The results of analyses of the edible portions of 43 varieties of Bengal fresh water fish regarding their water, protein, fat, calcium, phosphorus, total and ionisable iron content have been reported earlier. In continuation of the above work a large number of samples obtained from 11 more varieties of fish (of which 4 varieties were sea fish obtained from the Bay of Bengal) have been investigated. The four species of sea fish investigated did not have any special features regarding the aforesaid constituents.

It may be useful here to give in the following tables a list of those varieties of fish, which have hitherto been found richest in the different nutritive components, *viz.*, fat, protein, calcium, phosphorus, total and ionisable iron, copper and nicotinic acid. It may be noted that *Mahasole*, *Hilsa*, *Magur*, *Bata* and *Chela* have been so far found to be the richest sources of protein, fat, nicotinic acid, ionisable iron and copper respectively, *Chela* having an unusually high content of copper. *Parsey* seems to be the richest source of both calcium and phosphorus.

Figures are given per 100 *g. of fresh raw fish.*

Bengali name.	Zoological name.		Bengali name.	Zoological name.	
	Protein in gs.			*Calcium in gs.*	
Mahasole	Barbus (Tor) tor	25.2	Parsey (small)	Mugil parsia	1.05
Singhi ..	Saccobranchus fossilis	22.8	Bata (small)	Labeo bata	0.79
Hilsa ..	Clupea ilisa	21.8	Singhi	Saccobranchus fossilis	0.67
Amlet ..	..	20.8	Parsey (big)	Mugil parisa	0.65
Folui ..	Notopterus notopterus	19.8	Rohu	Labeo rohita	0.62
	Fat in gs.			*Phosphorus in gs.*	
Hilsa ..	Clupea ilisa	19.4	Parsey (small)	Mugil parsia	0.70
Dhain ..	Silonia silundia	12.1	Singhi	Saccobranchus fossilis	0.65
Pangas ..	Pangasius pangasius	10.8	Bhole	Scianca coitor	0.58
Sarputi ..	Barbu sarana	9.5	Lata	Ophicephalus punctatus	0.53
Bhangar ..	..	8.8	Boal	Wallago attu	0.49
	Total iron in mgs.			*Ionisable iron in mgs.*	
Mahasole ..	Barbus (Tor) tor	3.83	Bata (small)	Labeo bata	1.01
Parsey (big)	Mugil parsia	2.23	Singhi	Saccobranchus fossilis	0.97
Chital ..	Notopterus chitala	2.98	Chela	Chela bacaila	0.96
Singhi ..	Saccobranchus fossilis	2.26	Palmplate	Pampus argenteus	0.83
Hilsa ..	Clupea ilisa	2.13	Mahasole	Barbus (Tor) tor	0.80
	Copper in mgs.			*Nicotinic acid in mgs.*	
Chela ..	Chela bacaila	0.510	Magur	Clarius batrachus	1 02
Lady vendi	Sillags sihasna	0.230	Lata	Ophicephalus punctatus	1.00
Bata (small)	Labeo bata	0.170	Boal	Wallago attu	0.98
Chital ..	Notopterus chital	0.165	Dhain	Silonia silundia.	0.91
Koi ..	Anabus tostudinous	0.162	Katla	Catla catla	0.82

These figures enable a choice of fish diet having the required quantities of fat, protein and other nutritive constituents.

EFFECT OF COOKING ON THE BIOLOGICAL VALUE OF FISH PROTEIN.

That in the process of cooking the proteins very often undergo denaturation, hardening and drying, which to some extent affect their digestibility by proteases as studied under *in vitro* conditions has already been reported. In continuation of the above work, the biological values of a few species of fish after different processes of cooking have been investigated by the rat growth method. The best results regarding the biological values of these fish proteins were obtained with underdone fish as compared with raw, fully cooked or fried fish. These findings are in agreement with the previous digestibility experiments *in vitro*. In the case of Hilsa fish (*Clupea ilisa*), however, the biological value of the protein was found to be the greatest with fried fish and least with raw fish. *Hilsa fish* has a large content of fat, which has a higher melting point than the fat of some other fish. In general, fats having a higher melting point have a lower digestibility. It is possible that the loss of fat from *hilsa* fish on frying may have something to do with the better nutritional availability of the fried hilsa fish.

THE METHOD OF ESTIMATION OF AVAILABLE IRON IN FISH TISSUE.

It has been reported previously that Hill's method of estimation of ionisable iron would give rather low values for available iron in some tissues and a modification of the method has been described by which the tissue is previously digested with pepsin or treated with 10% acetic acid before being treated with the dipyridyl reagent. Further investigations have shown that this method would give more accurate values when applied to all animal tissues so far studied.

THE NATURE OF IRON IN FISH TISSUE.

It is obviously of importance to throw further light on the nature of this iron in fish tissue and to investigate by direct experiments how far this iron is available both in relation to (1) the formation of the total iron reserve of the body and to (2) its mobilisation for the actual building of haemoglobin.

This iron has been found to be non-haemin iron and to be an iron-copper-nucleoprotein complex, which has been obtained in a fairly pure state. The presence of copper in the complex is particularly noteworthy in view of the observations of various workers on the role of copper in haemopoiesis. By experimenting with rats rendered anaemic on a milk diet it has been found that this complex leads to the formation of the normal reserve of iron in the body and to its utilisation for heamopoiesis. There are reasons to think that this iron-copper-nucleoprotein complex is probably a specific precursor for haemoglobin building.

13. Studies on human nutrition with Indian diets under Dr. K. P. Basu at the Dacca University, Dacca.

Metabolic experiments performed on human subjects with some typical Indian diets, reported in the previous year, have pointed out that while these diets were fairly good in meeting the requirements of energy, protein and other minerals, they were, unless supplemented with milk, deficient with regard to calcium. The great value of betel-chewing in supplying available calcium was also pointed out.

Calcium.

(*a*) *Calcium from fish bone*—The search for cheap and rich sources of available food calcium was continued. Fish forms an important ingredient in the rice-eater's diet in certain parts of India and when small fishes are incorporated in the diet the soft bones are invariably taken. It has been found that calcium from such bones is utilised in metabolism and can meet the deficiency of this element in typical dietaries. For example, a subject living on a rice diet containing 226 mg. of calcium and exhibiting a negative balance of -95 mg. retained 145 mg. of calcium on the addition of 70 gm. of small fish. Similar results were obtained with other experimental subjects.

(*b*) *Calcium from Cucurbita pepo*—Again, in the course of our search for a rich source of calcium among the common green vegetables of Bengal, it was observed that the leaves and supporting stalk of *Cucurbita pepo* is the richest known available source of calcium. Examination of at least twenty samples of the leaves revealed that the calcium content of the leaves varied between 240-300 mg. per 100 gm. of moist leaves (moisture content 90%) while milk contains about 120 mg. of calcium per 100 c.c. Moreover, these leaves are free from oxalic acid, whose presence in vegetables is known to exert a deleterious effect on the utilisation of calcium. Metabolic experiments with these leaves were carried out on two adult males and on one pregnant woman during the latter part of her gestation. G.C.D. (60 kilos) on milk diet, containing 651 mg. of calcium retained 278 mg. calcium; when milk was replaced by *Cucurbita pepo* leaves of equivalent calcium content, he retained 384 mg. Again he retained 460 mg. on milk diet and 488 mg. on *Cucurbita pepo* leaves diet, both the diets containing almost similar amounts of calcium (933 mg.). Another subject H.P.D. (45 kilos) retained 265 mg. calcium on milk diet and 306 mg. on *Cucurbita pepo* diet, the diets containing about 500 mg. of calcium. When equal amounts of calcium (750 mg.) from milk or *Cucurbita pepo* leaves were incorporated in diets (calcium content 1250 mg.) fed to a woman (60 kilos) from 31st to 38th week of her pregnancy, she retained about 450-500 mg. of calcium per day on both diets. These results, therefore, show that the calcium from the *Cucurbita pepo* leaves is in no way inferior to, if not better than the calcium of milk as regards availability and utilisation.

Magnesium.

The magnesium content of a large number of foodstuffs *viz.* cereals, pulses, fishes, leafy and non-leafy vegetables has been determined. The magnesium content of rice is about 40 mg., of wheat 56 mg., of sago 8 mg., of fishes about 27 mg. (moist basis), of non-leafy vegetables between 40-51 mg. and of leafy vegetables between 35-85 mg. per 100 gm. of the foodstuffs.

Metabolic experiments on human subjects have been continued. In our previous investigations with typical rice and wheat diets the magnesium content was rather high (0·40-0.60 gm.) and the subjects always maintained a high positive balance. In experiments carried out this year the daily magnesium intake was reduced to 0.156-0.354 gm. per day and even then the subjects maintained positive balance and showed a total elimination of 0.102 gm. of the element. It is obvious that the minimum requirement of magnesium must be even less than this.

The availability of magnesium in certain varieties of leafy and non-leafy vegetables has been studied.

Effect of vitamins on mineral metabolism.

In last year's report it was pointed out that the intake of an excess of calcium decreased the retention of iron and copper. Since vitamin D affects the metabolism of calcium, experiments were conducted to find out the effect of vitamin D on the utilisation of iron and copper. It has been found by direct metabolic experiments on human subjects that the administration of high doses of vitamin D depresses the retention of iron and copper. Superimposition of excessive dose of vitamin D (4000 units) to a rice-fish diet decreased the metabolic balance of iron from +5·67 mg. to —6·20 mg. and that of copper from +2·03 mg. to +0·01 mg.

Intake of 50 mg. of vitamin C increases the retention of iron from +4·06 mg. to +21·36 mg. while the intake of vitamin A (2000 units) changes the balance of iron from a high positive to a highly negative value.

Experiments were also undertaken to find the effect of all and the individual factors of vitamin B complex. Superimposition of 10 gm. of marmite (yeast product), 10 gm. autoclaved marmite and 20 mg. of lactoflavin per day increased the retention of iron from +2·18 mg. to +19·41 mg., from +5·34 mg. to +9·86 mg. and from +6·45 mg. to +21·49 mg. respectively. Vitamin B_1 (Benerva-Roche) and nicotinic acid did not make any appreciable change in the utilisation of iron.

Lactoflavin autoclaved marmite and vitamin C increased whereas nicotinic acid decreased the utilisation of calcium.

Experiments are in progress to find out the effect of vitamins on the utilisation of other mineral elements.

14. Enquiry into calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

The enquiry has dealt mainly with the investigation of the alterations in the concentration of certain blood constituents due to the onset of rickets diagnosed clinically in infants and children and induced experimentally in animals.

Experimental rickets.

Six puppies of 4 to 5 weeks from two litters were equally divided into two groups. The experimental group received the vitamin D deficient diet consisting of cooked polished rice, white bread, and skim milk powder. The control groups received 400 I. U. of vitamin D per dog per day in addition to the basal diet. Red palm oil, marmite and ascorbic acid were fed to all the dogs in adequate amounts to meet their needs, for the vitamin A, B (group) and C respectively.

Blood from each dog was drawn from the jugular vein once a fortnight, and analysed for (1) calcium, (2) acid soluble phosphorus (inorganic and total), (3) total protein, (4) albumin, globulin, (5) total base and chlorides. The pH was determined on blood drawn in a paraffin syringe and delivered under a layer of paraffin in small tubes containing potassium oxalate. The dogs were weighed every fortnight and X'ray photographs of the wrist joint taken at intervals. The first definite radiological sign of rickets was seen in the experimental group after 16 weeks on the vitamin D deficient diet.

From the results of analyses it appears that the blood constituents which show variations in concentration are total calcium, albumin, globulin and to some extent inorganic phosphorus. The changes in the concentrations of total calcium, albumin and globulin should be considered highly significant in the light of the fact that the total protein remains unchanged. McLean and Hastings, who considered that calcium existed in blood mainly in two forms *viz.* protein bound calcium and Ca^{++} ions formulated the equation where

$$\frac{[Ca^{++}].\ [Prot.^{--}]}{[Ca\ Prot.]} = K.$$

the amount of total calcium invariably corresponds to calcium ionized plus protein bound calcium. It is evident, however, that the changes in calcium or protein concentration singly will affect the amount of the ionized calcium. In the present series of experiments, although the protein concentrations remained within the range found in the blood of control dogs (6·03 to 6·39 gms. per cent), the total calcium concentration showed an appreciable decrease with the onset of rickets. The range in the control group is 2·63 to 2·84 milli-Mols, that in rickets being 2·36 to 2·53 mM. per Kg. of serum water. The amount of $[Ca^{++}]$ calculated on the basis of McLean & Hastings' equation, showed a decrease from 1·40 to 1·47 mM. in control dogs to 1·12 to 1·17 mM. in rachitic animals.

Clinical rickets.

Several samples of blood from cases treated as rickets have been analysed. To take only one series, out of 20 cases, 13 showed radiological signs of rickets to a varied degree while 7 were radiologically negative although the blood calcium was low.

In the following table are grouped the observed values for calcium, total protein and the calculated values for Ca^{++}.

Number of cases.	Total calcium mM. per Kg. of serum H_2O.	Total protein per 100 c.c. serum.	Ca^{++} mM. per Kg. of serum H_2O.
5	1·85 to 2·11	6·69 to 7·84	0·79 to 0·82
6	2·11 to 2·37	6·49 to 8·29	0·86 to 1·06
5	2·37 to 2·64	6·92 to 8·87	0·92 to 1·06
4	2·64 to 2·95	7·25 to 8·13	1·06 to 1·18

These observations cannot be reconciled with the findings of McLean and Hastings, who showed that in clinical rickets ranging from mild or doubtful to marked active stage of the disease the $[Ca^{++}]$ remained within normal limits. A study of their data reveals the fact that in their series of rachitic cases the concentration of protein and total calcium in serum was within normal limits and that consequently, the $[Ca^{++}]$ was unaffected.

The concentration of Ca^{++} in serum is of utmost importance. Normally the body attempts to maintain the level of Ca^{++} in serum within a very narrow range, in cases of gross disturbances however, it may not succeed in doing so. Further work is in progress which is expected to throw light on the relation between rickets, the $[Ca^{++}]$ of serum and the effects of the alteration in the latter on normal calcification.

15. Enquiry into malnutrition of Bengali boys with reference to vitamin A deficiency under Professor N. M. Basu at the Presidency College, Calcutta.

The following investigations were undertaken :—

Assessment of vitamin A deficiency amongst Bengalis.

The new biophotometer method for assessing the vitamin A status of human beings which was devised in these laboratories last year, has since been confirmed by Hecht in America. This technique is being used in these laboratories for routine work. An additional number of apparently normal subjects and patients with definite eye lesions have been tested. Simultaneously, some of these patients were given daily for several weeks large doses of vitamin A and the effect on the various eye lesions studied. All cases of impaired dark adaptation, xerophthalmia and night blindness showed a positive improvement, while pigmentation of the conjunctiva and Bitot's spots were not cured. It is concluded that these two eye lesions are probably caused by multiple factors.

The minimal requirements of vitamin A for human beings.

After having ascertained the optimal requirement (5000 I. U.) of vitamin A experiments were completed to determine the minimal requirements. It is concluded that the minimal requirements for boys is 4000 I. U. of vitamin A per head per day and that for the adults 3000 I. U.

Vitamin A storage in the liver under different conditions of diet.

Experiments were completed to study the variations in vitamin A storage in the liver of rats under varying conditions of protein and carbohydrate intake. The results obtained lead to the following conclusions :—

An excess of carbohydrate intake at the expense of protein (which is the prevailing habit amongst the poorer Bengalis) was found to be detrimental to the deposition of vitamin A in the liver. This fact ought therefore to be taken into account in any measure of reform in the diet of persons suffering from vitamin A deficiency.

4. Experiments are in progress to study the influence of vitamin A on the following :—(*i*) detoxicating function of the liver, (*ii*) storage of glycogen in the liver, and (*iii*) sulphur metabolism.

5. Chronaxemitry :—

Investigations are in progress to ascertain if there are any variations in the chronaxie of leg muscles of rats due to vitamin A deficiency. Experiments are also being performed on human beings by the method of Bourgninon if the chromaxie of rod and cone vision is altered in vitamin A deficiency. As these experiments require very careful manipulation, the progress of this work is necessarily slow.

16. Enquiry into malnutrition of Bengali boys with reference to vitamins B and C deficiency under Professor N. M. Basu at the Presidency College, Calcutta.

The following investigations were undertaken :—

I. The effect of vitamin B_1 on the detoxication processes in the liver of rats has been studied and was found that in vitamin B_1 deficient animals there is a tendency for diminution of the urinary glucuronic acid excretion after the introduction of a toxic substance in the body.

It was noticed that after damaging the liver by oral administration of CCl_4 (0.176 c.c. daily per kg of body weight for a whole week) and then administering chloral hydate (200 mg. per day per kg of body weight for one week), the control group excreted, on the average, 0.7 times more of glucuronic acid than the deficient one. As the deficient rats are more susceptible to these toxins, some of them succumbed before the experiment could be completed. Thus these experiments were performed on a comparatively smaller number of animals and would hence require further work for confirmation.

II. The influence of vitamins B_1 and C on the effects of adrenaline on a strip of intestine of rabbit in calcium deficient, normal, calcium excess and potassium deficient Ringer has been observed. It was noted that

(*i*) In normal and calcium deficient Ringer vitamin B_1 (100 μg in a bath of 200 c.c.) tends to neutralise the effect of adrenaline (1 drop of 1 : 100,000 in the bath). In calcium excess Ringer this effect of B_1 was not observed.

(*ii*) The addition of 10 mg. % vitamin C to normal Ringer did not affect the contractions but when added to calcium deficient Ringer the contractions increased in amplitude. Vitamin B_1 did not affect this effect of Vitamin C.

(*iii*). In potassium deficient Ringer vitamin C (10 mg %) has a slight stimulating effect on the contractions but when added with vitamin B_1 (10 μg. in 200 c.c.) there is marked inhibition of the contractions. On the addition of 1 drop adrenaline (1 in 100,000) to the same bath the inhibiting effect of vitamin B_1 was neutralised and slight augmentation was noticed.

Vitamin B_1 alone had no effect on the contractions.

III. With a view to investigating if chronaximetry can be used for assessing avitaminosis B_1, the chronaxie of vitamin B_1 deficient and normal rats was ascertained in several groups of animals. It was found that the chronaxie was definitely and appreciably higher in deficient rats as compared with that of control rats. Further observations are necessary with regard to changes in chronaxie during vitamin B_1 deficiency and subsequent restoration to normal health by vitamin B_1 feeding, as compared with similar possible changes in animals caused by deficiency of other vitamins or minerals.

IV. The chronaxie of 50 persons was examined at different periods of the day and was found to be generally below 1.0. It was also noticed that the chronaxie, as a rule, is higher after meals than before.

V. Breads prepared from whole wheat 78.1%, fried gram-powder 15.6%, Nacl 1.0%, calcium lactate 0.06% and dried and powdered spinach 5.24%, when tried on rats for a period of over two months promoted satisfactory growth (10 g per week, on the average) of the animals.

These breads kept well for nearly a month during the hot season, but they became a little hard. If these breads could be baked, this hardening could be prevented.

From the very satisfactory results obtained with this bread it is strongly recommended that they are adopted for use by soldiers during emergency and by the poor people of this country. As this bread is quite tasteful and as different preparations of wheat and dal are used for consumption in different parts of India, this type of bread is expected to be readily accepted by the people.

17. Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lt.-Col. E. W. O'G. Kirwan, I.M.S., Professor of Ophthalmic Surgery, Medical College, Calcutta.

The work is being carried out in the Eye Infirmary, Medical College Hospitals, Calcutta, and in the districts of Bengal in the travelling eye dispensaries of the Association for the Prevention of Blindness, Bengal.

In the Eye Infirmary 488 cases have been investigated and treated for vitamin A deficiency. Of these 125 cases were of keratomalacia from its earliest stage to the severest ones of blindness. Twentyseven cases of keratomalacia came in very late stages when the eyes were completely lost; the treatment therefore could only be carried out for their general health. Of these keratomalacia cases, 93 (*i.e.* 74.4%) were below the age of five years.

Out of 78 cases of night blindness it has been noted that 66 were due to vitamin A deficiency, 3 cases were due to diseases of the retina or optic nerve and 9 cases could not be followed up.

Shark liver oil procured from Madras as medicinal fish liver oil and from Calcutta as Avitol are being tried on clinical cases. The therapeutic values are under observation.

The results of parenteral administration of vitamin A concentrate are also being observed on the following two preparations (*i*) 'Prepalin' of Glaxo Laboratories, 1 ccm. containing 100,000 i.u. of vitamin A and (*ii*) vitamin A concentrate from shark liver oil prepared in the Coonoor Research Laboratories and kindly supplied by Dr. Aykroyd. One hundred and ten cases have been treated with parenteral vitamin A concentrate. Very good results have been noted in 66 cases, 2 cases are still under observation. Results could not be followed up in 42 cases.

Field work amongst jute mill workers, jail prisoners, school boys and people of rural areas were carried out by the clinical worker of the enquiry in the districts of Khulna and Hooghly in collaboration with the travelling eye dispensaries of the Association for the Prevention of Blindness, Bengal. Five thousand one hundred and thirtyone jute mill workers, 210 jail prisoners, 945 school boys, 94 patients of the travelling eye dispensaries were examined.

In Hooghly 162 had clinical evidence of vitamin A deficiency as noted from the ocular signs *e.g.* pigmentation xerosis and Bitot's spots of the bulbar conjunctiva in the palpebral fissure or dermal change, *e.g.*, phrynoderma, of these 61 cases were tested with the bio-photometer to note the vitamin A content of the system, 59 cases (*i.e.*, 96.8%) were found to be normal.

The Principal of the Medical College, Calcutta has kindly sanctioned the purchase of a bio-photometer. It was made locally by the Applied Physics Department of the Calcutta University. It is worked both by house current and battery and is satisfactory. It will now be utilized for the investigations of the enquiry in place of the old one, kindly lent to us by Dr. G. Sankaran, Professor of Bio-chemistry and Nutrition, at the All-India Institute of Hygiene and Public Health, Calcutta.

18. Enquiry on the nutritive value of fish liver oils obtained from fish along the Bombay sea coast under Professor S. P. Niyogi at the Seth G. S. Medical College, Bombay.

Seasonal variation in the yield and the vitamin A content of fish liver oils.

The results of the investigation which was completed at the end of April, 1941 are presented in table I. Out of the five types of fish selected complete information about *Ravas* could not be obtained as this fish was not available during the monsoon months. Some constants of the fish liver oils have also been determined and are given in table II. The figures represent the mean of four observations on fish livers collected at monthly intervals.

The yield of the oil from the livers varied from month to month in the case of each fish. The vitamin A content also showed enormous variations. The yield of the oil and its vitamin A content bore no relation to one another.

Biological assay of vitamins A and D in fish liver oils.

(1) *The assay of vitamin A*—A sample of *Mushi* liver oil which gave the value of 7821 I. U. per g. of oil by the tintometric method was selected for assay by the growth method. 48 rats were equally divided into six groups and fed with a vitamin A deficient diet. The rats from three groups were given orally a supplement of 0.223, 0.446 and 0.892 mg. of mushi oil per rat per week. The animals in the control groups received doses of reference cod liver oil corresponding to 3.5, 7 and 14 I. U. per rat per week for three weeks. The curves of response were drawn from the observed increase in weights. The value for vitamin A was then calculated and found to be 7440 I. U. per g. of the oil.

(2) *The assay of vitamin D*—The bone ash method was followed for the determination of this vitamin. 56 rats were equally divided into seven groups and were kept on a rachitogenic diet. The rats in three groups received increasing doses of the *mushi* oil, the animals in the other three groups receiving doses of the standard oil. One group served as the negative control. At the end of the assay period the rats were killed, the femora removed, cleaned, extracted with alcohol and ether and dried. The dry weight of the bone and the ash were determined. The vitamin D was estimated by comparing the curves of response of animals in the two groups. Mushi liver oil was thus found to contain 98 I. U. of vitamin D per g. of oil.

Similar investigation on other fish liver oils is in progress.

Table I.

Seasonal variation in the yield and vitamin A content of the fish liver oils.

Month.	Mushi (Scoliodon sorrakewah).		Wagli (Dasybatus imbricatus).		Ghol (Sciaena miles).		Shengti (Macrones gluilo).	
	Oil per cent.	Blue units per g.	Oil per cent.	Blue units per g.	Oil per cent.	Blue units per g.	Oil per cent.	Blue units per g.
1940								
May ..	49.80	8853	29.17	300	16.50	7843	7.85	9596
June ..	61.40	6824	25.54	490	17.26	7056	7.40	6892
July ..	23.7	9072	25.75	218	7.04	9703	4.97	7953
August ..	63.46	5530	31.70	332	5.07	17140	2.70	8871
September ..	47.81	5575	26.60	1208	4.26	19385	1.34	13000
October ..	60.90	2627	27.30	1987	Not found		1.34	14290
November ..	53.46	2132	25.80	714	4.89	11111	5.20	7994
December ..	48.60	2531	26.34	686	7.36	9802	8.20	7691
1941								
January ..	41.36	6437	25.46	634	10.32	8644	9.20	7235
February ..	39.63	6563	23.36	588	9.63	8932	7.65	7839
March ..	42.60	6324	22.86	487	12.60	7364	8.76	7832
April ..	43.66	6250	23.46	474	14.62	7091	8.85	8090

Table II.

Some constants of the fish liver oils.

Name of fish,*	Acid value.	Saponification value.	Iodine value.	Nonsaponifiable matter p.c.
Mushi ..	2.40	203.0	134.0	2.87
Ghol ..	1.35	184.3	136.0	5.00
Shengti ..	1.03	176.0	151.6	4.33
Wagli ..	2.89	190.4	122.7	2.30

*N.B.—For Scientific names refer to Table I.

19. Studies on metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

The enquiry started on the 19th June 1941 with a staff of a biochemist and a laboratory assistant. The latter post was not filled for the first two months. During these months the biochemist underwent a training in—

(1) Use of the spectrograph and the rotary sector in absorption Spectroscopy.

(2) Estimation of vitamin A by the spectrophotometric and colorimetric methods.

(3) Estimation of the minimum amount of vitamin A in oils and margarines associated with substances giving rise to irrelevant absorption.

(4) Preparation of vitamin A concentrates from fish liver oils and margarines by the method of saponification, special care being taken so that the loss of vitamin A was reduced to the minimum.

(5) Considerable library work and consultation of journals useful for future work.

The vitamin A content of a fish liver oil was determined several times both in the oil and in the non-sap with an idea to know what percentage of vitamin A is lost during saponification. Although the technique has been improved a good deal there is a difference in the extinction coefficients at λ 3280 of the original oil and of the non-sap. This may be due to the loss of vitamin A and to the presence of substances other than vitamin A. Many suggestions, such as oxidation of vitamin A by some organic peroxides or ozone are suggested and are being studied further.

Vitamins A and D were estimated in many samples of margarines supplied by the Military Food Laboratory, Kasauli. In these samples the potency of vitamins A and D was very poor. So, large quantities of margarines were taken and concentrated extracts were prepared for spectrophotometric examinations. But the difficulty was the pigments and other ether soluble

impurities of the margarines also became concentrated which were responsible for absorption of the ultra-violet light in the region we require. Therefore, an improved technique was followed which is as below. Some quantity of margarine is saponified with NaoH and extracted with ether in the usual way. Same quantity of the sample of margarine is saponified with sodium peroxide which oxidises vitamin A only and then extracted with ether. This second extraction which is supposed to contain no vitamin A is placed in the compensating cell of the Spectrophotometer. Thus the absorptions due to impurities are eliminated and the resultant absorption corresponds to vitamin A only. Further tests are being carried on and the results so far obtained were very encouraging. Vitamin D content of these samples of margarines were also estimated biologically.

At the suggestion of Col. E. Cotter, the Public Health Commissioner, a series of experiments were conducted to estimate the vitamin A content of ghee left after frying '*puris*' and also of the ghee absorbed in the '*puris*'. On examination it was found that practically no vitamin A was present in the cooked ghee which was heated to about 200°C-250°C for frying '*puris*' although the potency of vitamin A in the original ghee was as high as 25 I. U./gm. Since this work is very important further experiments are being carried on by frying fish, brinjals, etc.

In addition to the studies of various methods of estimation of vitamin A and their improvements the facilities of the enquiry were made use of in giving demonstration to D.P.H. students on the assessment of vitamin A by the spectrophotometric and colorimetric methods.

20. Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay.

The object of the enquiry is to see how far the various tests of physical fitness in use in England and America are applicable to Indians and to work out the standards for Indians in these respects. It appears from the literature that so far no such data have been worked out in this country.

The actual work was begun in July 1941. It was planned to record observations on three groups of tests. The subjects were mostly medical students and the laboratory staff. Observations have been done on 199 males and 40 females so far. A brief summary of the observations is given below:

I. Circulatory tests.

These are mainly in use in America for the recruitment of candidates to the American Air Force and for determining staleness and fatigue among members on active services.

(*i*) The Schneider test—In this the pulse rate and the systolic blood pressure are observed in the recumbent and the standing positions and their variations under these conditions noted. The pulse rate variation after a definite amount of exercise (stepping up 5 times in 15 seconds on a stool 19 inches high) and the time of return of the pulse to the normal standing level are also noted thereafter. Each of these measurements scores certain points (given in Schneider's score table) and the total number of points scored by

the subject grades his physical fitness. In the cases observed here the scores varied from —4 to +17 in the males and from +3 to +16 in the females; nearly 88% of the former and 78% of the latter had scores of 8 and above which is given as the minimum score for fitness for the American males.

(*ii*) The Crampton 'blood ptosis' test—In this the reactions of the pulse rate and the systolic pressure to the change from the recumbent to the standing posture are taken into account. From these Crampton has worked out a percentage scale of physical fitness by adjusting the changes in pulse rate in terms of corresponding blood pressure variations. Fifty is taken as the minimum index of fitness for males. In the cases observed here the index lay between 40 & 95 in the males and between 40 & 75 in the females; 95% of the former and 90% of the latter fell above the minimum of 50.

(*iii*) The McCloy test—This employs a formula for scoring, the two variables employed being the standing diastolic arterial pressure and the standing pulse rate. The minimum index given is zero for males. In the cases observed here the index varied from —26 to +29 for males and from -30 to +19 for females; 47% of the former and 35% of the latter gave indices of zero or above.

II. Respiratory tests.

These tests are mostly in use in England for the recruitment of candidates to the Royal Air Force.

(*i*) The Breath-holding test—The seated subject is instructed to expire once as deeply as possible, then to inspire as deeply as possible and then to hold the breath as long as possible. The minimum time given for males in England is 45 seconds. Here the time varied between 25 and 180 seconds for males and between 6 and 68 for females; 78% of the former and 30% of the latter could hold the breath for 45 seconds and above.

(*ii*) The Expiratory force test—The subject is asked to blow steadily the column of mercury in a manometer as high as possible. The test is said to be a measure of the tone of the abdominal wall. The minimum height blown by males in England is 80 mm. Here the height varied from 20 to 200 mm. in males and from 10 to 110 mm. in females; 71% of the former and 10% of the latter could raise the column to 80 mm. and above

(*iii*) The Persistence test—In this the mercury column is blown to half the height of the Expiratory Force Test and held there as long as possible without breathing. The nose is clipped. In England the average sustaining time for males is given as 40 seconds. Here the time varied between 6 & 64 seconds for males and between 0 & 47 seconds in the females; 26% of the former and 10% of the latter had a timing of 40 seconds and above.

The test was also done with the column of mercury kept at 40 mm. height.

(iv) Vital capacity—This is measured with a wet spirometer. The minimum for males in England is given as 3000 c.c. Here it varied from 2100 to 2500 c.c. in males and 1400 to 3050 in females; 45% of the former had a reading of 3400 c.c. and above.

(v) Flack-Woolham index of fitness—This index is calculated from a formula in which the expiratory force, the persistence test time and the breath-holding time are taken into account. This index is being worked out.

III. GENERAL DEVELOPMENT OF THE BODY AS INDICATED BY CERTAIN MEASUREMENTS.

For this the standing and sitting height, the weight, the chest (inflated, deflated and resting), and the abdomen were measured. From these the surface area, the mean chest, the trunk area (sitting height × mean chest), and the nutritional indices (Von Pirquet's, Rohrer's, and Davenport's) are being calculated.

The work is in progress and when a substantial number of observations are done it will be possible to work out the standards applicable to the Indian civilian population for all these tests and measurements. It will also be possible then to correlate the tests with such factors as age, sex, and the measurements.

21. Enquiry on the formation functions and variations of plasma proteins in health and disease under Dr. N. C. Datta at the Grant Medical College, Bombay.

An understanding of the physiology of plasma proteins is important in the study of numerous functions in which healthy and pathological condition merge into one another. Study of plasma proteins may give useful informations as to the phase of many diseased conditions. Investigations have been carried out in about 300 clinical cases in order to study the range of changes usually observed in the proteins of the plasma in various pathological conditions and their significance.

PLASMA PROTEINS IN NORMAL INDIVIDUALS.

Normal controls for plasma proteins have been based on a few observations. Different workers agree in the main as to the average of each of the individual proteins but there would appear to have a wide range of margin between the lowest and the highest value found normally. Since in India malnutrition is an important cause of disease, it was thought essential to establish the normal range of variation and average values for plasma proteins, as they are of importance for the interpretation of the findings in patients.

The results recorded for 35 healthy males and 12 females may be compared with those of Salvensen (1926), the largest published series based on 32 determinations.

Number of observations	Total protein	Albumin	Globulin	Ratio
15 medical students ..	7.16	4.94	2.12	2.29
20 normals from other sources ..	7.00	4.69	2.31	2.00
Average of 35 normals ..	7.08	4.81	2.21	2.14
Salvensen (1926) ..	7.00	4.44	2.58	1.72
Average of 12 normal females ..	6.95	4.71	2.24	2.11
Average of 12 normal pregnancy	6.56	3.55	2.99	1.23

A significant fact observed in the present series of normal cases was that the globulin content of healthy medical students was lower in every case than that derived from other sources.

Normal females :—

There is some evidence in the data of Salvensen (1926) that the globulin of adult females was higher than that of males. In the present enquiry no significant difference between the two sexes has been observed so far. An increased globulin content was observed in most of the cases of normal pregnancy in spite of the fact that the total protein was always less than the normal.

Malnutrition and plasma protein.

Investigations on 8 cases of nutritional oedema showed that the total protein was much below the normal average, being only 4.7 per cent. The deficiency was entirely due to the albumin fraction, a value of 2.5 per cent based on 15 determinations being obtained. In a few cases of oedema of a severe type in children the globulin was found below to be normal. The oedema of malnutrition in children was found to be successfully eliminated by dietetic treatment, while that in adults appeared to be more resistant to treatment.

Anaemia.

The present series comprising of more than 50 cases of different types of anaemia showed a general reduction of total protein from its normal average of 7.0 to 5.5 per cent, the albumin fraction being subject to reduction. Twenty cases of anaemia with oedema had an average albumin content of 3.0 per cent. In 5 cases out of 50 cases the plasma protein was found to be within normal limits.

Nephritis.

Twentyfive cases of nephritis have been studied so far, 12 cases being in children. The protein deficiency is entirely in respect of the albumin fraction. A value of 2.5 per cent of albumin was obtained in 12 out of 25 cases. Values lower than this were also obtained in a few cases. In a child of 10 years, the albumin was only 0.7 per cent out of a total of 2.35 per cent. Eighteen cases among the 25 showed increased globulin content indicating that the disease was either accompanied or followed by some infection. Four cases of nephritis are now under observation. The amount and nature of the loss of protein in the urine is being investigated with a view to finding out the extent to which protein loss accounts for the deficiency of plasma proteins.

Severe cutaneous burns.

Plasma proteins and the amount of protein in blister fluid have been determined in 2 cases of severe burns.

Miscellaneous diseases.

This series includes 16 cases of cardiac failure with and without oedema and 50 cases of different diseases. Half the number had higher globulin content.

Changes in plasma proteins in different types of infections.

It is well known that there is an increase of globulin in many diseases. Some authors consider that this occurs at the expense of albumin. This view has often been contradicted.

The results of observations on 30 cases of unidentified diseases which were sent for Wasserman reaction, when compared with those of 8 cases of tetanus investigated so far, brings out certain facts which appear to be interesting and significant.

Disease	Number of cases	W.R.	Total protein	Albumin	Globulin	Ratio
Unidentified	15	—ve	7.21	4.78	2.52	1.93
	12	+ve	7.22	3.68	3.53	1.02
Tetanus ..	8	..	7.53	4.53	2.12	1.50

The 15 cases with negative Wasserman had the plasma proteins within normal limits. In all the 12 cases with positive Wasserman there was an increase of globulin which seemed to have occurred at the expense of albumin. In cases of *tetanus*, the increased globulin was not associated with a decrease of albumin; the total protein appeared higher. A comparative study of the globulin content with a study of sedimentation rate in different infections is in progress.

Animal experiment.

In view of the recent interest in plasma-proteins for the treatment of surgical shock, experiment have been started from the point of view of origin, site of formation of plasma protein and the value of dietary protein in relation to the regeneration of plasma protein and their value in the treatment of oedema.

LEPROSY.

22. Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta.

IMMUNOLOGICAL STUDIES.

(*a*) *Mitsuda reaction*—The study of the Mitsuda reaction has been carried to a point and then abandoned since as the result of the work, a better immunological test was evolved. This test is described later. The study of the Mitsuda reaction gave the following results :—

(*i*) The bacillus has been shown to be the only antigenic element of lepromin.

(*ii*) A method for the preparation of standard lepromin almost free from nodular tissue has been evolved. One tenth c.c. of this standard preparation, containing about 1.5 million bacilli has given satisfactory results. The advantages of a standard preparation are obvious.

(*iii*) The Mitsuda test does not appear to be a test for specific allergy since positive results have been obtained in persons in whom the chances of exposure to leprosy infection are remote.

(*iv*) Several hundred tests have been done in cases of leprosy of all clinical varieties. In clinically typical lepromatous cases, 93% gave negative results; in clinically typical tuberculoid cases 95% gave positive results. In 7% of lepromatous and 5% of neural cases somewhat anomalous results were obtained and these could not always be correlated with abnormal histology of the lesions. With our present knowledge the significance and prognostic value of these anomalous results cannot be accurately assessed. In other clinical varieties of leprosy the results are less clear cut.

(*v*) The report that when a lepromatous case becomes inactive the lepromin test is, or becomes, positive has not been confirmed.

(*vi*) Repeated lepromin tests, have been done in 89 cases of leprosy some of neural and some of lepromatous type. In only a few cases has repeated testing been accompanied by an increase in the degree of reaction and even in these few cases, the increase cannot with certainty be attributed to re-testing. In some cases, the degree of reaction on re-testing was less. The reports of other workers in this matter have, therefore, not been confirmed.

(*b*) *Early reaction to lepromin*—As stated last year Fernandez' report of an early reaction of tuberculin type seen after the injection of lepromin in cases which show a definite late reaction, has been confirmed. Further work has been done with the following findings :—

(*i*) The early and late reactions to ordinary lepromin both in cases and in contacts are usually in agreement.

(*ii*) Fernandez' statement that the early reaction is caused by a soluble antigen has been confirmed; but his view that the early

and late reactions are caused by different antigens has not been confirmed.

(*iii*) The early reaction has been shown to be caused by a free soluble antigen and the late reaction by the same antigen liberated later by the slow breaking down of the injected bacilli. Thus the lateness of the Mitsuda reaction has been explained and the test has been brought more into line with other skin reactions.

(*iv*) The appearance of the early reaction resembles strongly that of an allergic skin test but since early reactions have been obtained in non-contacts, the reaction does not appear to be specific.

(*v*) The antigen responsible for the early reaction has been shown to be of protein nature. This protein has been isolated.

(*c*) *Reaction to protein antigen isolated from the Hansen bacillus*—A method has been evolved for obtaining from leprous material the leprosy bacillus free from tissue. By a combination of physical and chemical methods it has been found possible to isolate the different chemical fractions of the bacilli. Tests with these fractions have shown that protein is the only antigenic fraction, and that it produces an early reaction only. On the basis of this work a test has been evolved using as antigen the acid-precipitated protein of the bacillus. The test with this antigen is at least as sensitive as the Mitsuda test, and has great advantages over it.

No attempt has been made to demonstrate specificity of this antigen. It seems probable, however, that it will not be specific. By extracting the bacillus with different solvents, however, three different protein fractions have been isolated, and all of these are antigenic in cases of leprosy. There is a possibility that one of these fractions will be found specific.

It is believed that this new test is of importance. The Mitsuda test takes a month to read and, if positive, causes nodulation and sometimes ulceration. The new test is read in 24 hours, the reaction does not persist, and causes no ill effects. The new test has explained some of the anomalies of the Mitsuda test and with further work may explain more. The new test should do much to facilitate further studies of the immunology of leprosy.

Epidemiological studies.

(*a*) *The Bankura investigation centre*—This centre, established in the year 1936, completed the original survey of a population of 10,000 in 1937. The records of the last four years have been brought up to date and analysed. The following is the summary of the main points of the analysis.

The number of cases in 1937 was 424 and the number in June 1941 is 422; the population has slightly increased during this period and the incidence has therefore slightly fallen. The proportion of lepromatous cases in 1937 was 22.64%; it is now 21.1%. During the four year period 59 cases have died; the death rate in cases of lepromatous type was 25% and of neural type 11%. During the four years 56 new cases have arisen in the area; of these 21% are of lepromatous type, and 61% are in children aged 14 and under. In 80% of the 56 new cases, there was definite evidence of contact with an infectious case, and in 50% there was an infectious case living in the same house. These facts indicate that contact with infectious cases early in life often in the home is responsible for most of the new cases arising in this area. The study of the

cases during the four-year period has shown, that, of the neural cases, many have remained stationary, while of lepromatous cases many have got worse, and 25% have died. Of the 328 neural cases in 1937 only two had become lepromatous by the year 1941. Both these cases are young children.

In spite of the absence of any effective isolation in the area there is at present no evidence that the disease is increasing.

(*b*) *Studies in other areas.* During the year epidemiological studies of the whole population of selected small areas have been carried out in the plains and in the hills of the Punjab, in the United Provinces, in Assam and in Orissa. These surveys have revealed marked regional variations not only in incidence, but also in the type and age distribution of cases. The main findings of these surveys are summarised as follows:—

	Incidence	Percentage of L. cases	Leprosy cases under 15 years
Plains of the Punjab . . .	Very low	33%*	24%*
Hills of the Punjab . . .	1.4%	53%	4%
Assam . .	1.8%	9%	11%
Bankura (Bengal) . .	3.9%	21%	19%
Pura Kulandar (U. P.) . .	0.9%	23%	7%

* Approximate figures.

The figures for Orissa have not yet been analysed. The possible significance of these differences is being studied but no definite conclusion has yet been reached.

Work on animals.

Our previous report that sulphanilamide and M & B 693 have a bactericidal action on the bacillus of rat leprosy *in vitro* has been confirmed. This has been demonstrated with M & B 693 in a dilution of 1 in 10,000 at incubator temperature, but not in the cool room. One in 1,000 sulphanilamide had the same effect but the experiment with 1 in 10,000 dilution was spoiled apparently by contamination. The results of these experiments were convincing; the experimental animals injected with material treated with M & B 693 and sulphanilamide remained free from leprosy, whereas the control animals injected with the same material untreated developed a generalised leprosy infection.

Attempts at the infection of splenectomised monkeys by repeated inoculation of leprous material have not succeeded. Monkeys inoculated eighteen months ago and reinoculated six months later, are still living and show no signs of the disease.

Sundry observations.

(*a*) A study of the seasonal variation of leprosy has been continued during the year. The figures for 1941 have not yet been analysed.

(*b*) Observations of the course of the disease have been continued in Calcutta and in Bankura. The findings made in Bankura have already been mentioned above.

(*c*) Histological studies in connection with the above work have been continued.

23. Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras.

The leprosy enquiry was continued as in previous years and covered the following lines of investigations :—

i. dietetic.
ii. therapeutic.
iii. clinical.
iv. child.
v. animal experiments.

Dietetic investigation.

It was stated in the report last year that the experiment on skim milk had to be commenced afresh as there was evidence that the factor of unconscious selection had not been completely eliminated. A fresh group of cases has now been under investigation for a year and the following table indicates the result :—

Table I.

From 1st October 1940 to 31st October 1941.

	Negative	Much improved	Improved	Stationary	Worse	Total	Average increase in weight	Average dose	Average period of treatment
Skim milk .. .	30	11	11	8	8	68	.5 lbs.	6½ c.c.	25 months
Percentage ..	44	16	16	12	12	100			
Control ..	30	7	10	14	7	68	5 lbs.	6½ c.c.	26 months
Percentage ..	44	10	15	21	10	100			

It will be noticed that the percentage of negatives (44%) is the same in both groups. This percentage appears to be high, but it is not in our experience unusually so, for we have evidence that this is not an exceptional percentage when the early lepromatous type is placed under intensive treatment. This percentage however must not be taken as a negative rate throughout the institution. In the above groups comprising 136 cases 98 were early leproma.

Wheat diet—Since the previous report there has been one failure on wheat diet, this was a boy who had an almost continuous febrile reaction, and it may be that when the leprosy condition is active, especially when associated with true lepra reaction of the chronic type, nerve pain and bone pain tend to persist so long as the reaction continues. Except for this reservation the general conclusions remain the same.

Some advanced lepromatous cases have been under wheat diet for more than two years and their disease has shown considerable improvement. An attempt will be made to ascertain whether patients who are placed on wheat diet over a prolonged period improve more satisfactorily than those who are on the usual diet. Up to now cases treated under 18 months have shown no

more satisfactory improvement in their leprosy condition with wheat diet than without it, but those treated for longer periods appear to have shown greater improvement; the number is too small to draw any conclusions.

Ragi and Rice Diet—No conclusions can be drawn as the ragi diet was too unpopular for its continuance. This illustrates one of the main difficulties of dietetic experiments.

THERAPEUTIC INVESTIGATION.

The experiment endeavouring to discover the optimum dose of hydnocarpus preparations continued throughout the year. The following tables summarise the result for the period from April 1941 to October 1941.

Table II.

Dose	Negative.	Much improved.	Improved.	Stationary.	Worse.	Total.
High (15-25 ccs.) ..	6	9	9	6	4	34
Percentage ..	18	26	26	18	12	100
Medium (10-15 ccs.) ..	11	7	8	6	5	37
Percentage ..	30	19	21	16	14	100
Low (1-10 ccs.) ..	8	13	12	21	9	63
Percentage ..	13	21	19	33	14	100

Average increase in weight – High .. 2 lbs.
Medium .. 1 lb.
Low .. 1 lb.

Table III.

Dose	Negative.	Much improved.	Improved.	Stationary.	Worse.	Total.
High (15-25 ccs.) ..	6	9	10	7	5	37
Percentage ..	16	24	27	19	14	100
Medium (10-15 ccs.) ..	16	7	9	7	6	45
Percentage ..	36	16	20	16	12	100
Low (1-10 ccs.) ..	4	13	9	17	9	52
Percentage ..	8	25	17	33	17	100

Average increase in weight – High .. 2 lbs.
Medium .. 1 lb.
Low .. 1 lb.

It appears that a greater number of those in the medium dosage (10-15 ccs.) group tend to become negative. Table II includes those who were on high, medium and low dosage at the time of assessment. In table III those who had reached the higher dosage group during the year were added, but at the time of assessment may have been in one of the lower dosage groups. It will be noted that the greatest number of cases are in the low dosage group; this is because certain patients who were originally selected for the high dosage were unable due to signs of reactions, to reach the selected dosage. We are of opinion that this confirms the traditional view that if a patient is unable to stand large doses of hydnocarpus preparations the prognosis is poor. The consolidated figures for the year October 1940 to October 1941 are given in Table IV.

Table IV.

Dose	Negative.	Much improved.	Improved.	Stationary.	Worse.	Total.
High ..	19	6	22	17	9	73
Percentage ..	26	8	31	23	12	100
Medium ..	35	7	17	9	5	73
Percentage ..	48	10	23	12	7	100
Low ..	26	8	12	18	9	73
Percentage ..	36	11	16	25	12	100

Between October 1940 and April 1941 thirteen in the high dosage group, nineteen in the medium dosage group and twentytwo in the low dosage group were discharged negative.

In order to ascertain whether the patients discharged were approximately at the same stage, those in the negative groups were analysed according to the stage of their disease into those who were fully positive at the commencement of the experiment and those who were partially positive. Three or more negative smears out of a total of fourteen smears were taken as partial positive. Table V gives the result of this analysis.

Table V.

	Fully positive.	Partial positive.
High ..	6	13
Medium ..	6	29
Low ..	4	22

These results suggest that the dosage which appears to be the best is between 10 and 15 c.cs. High doses above 15 c.cs. may be harmful. This work must be continued for a further period before conclusions less tentative than these can be drawn.

Trial of new remedies.—

(*a*) *Diptheria formol toxoid*—Fourteen cases have been given this remedy, the longest period a patient has been on the remedy is one year and the shortest period one month. The general conclusions are as follows:—

i. In certain cases toxoid may have been of help in relieving nerve pain, this relief however is temporary, in about four weeks there is a return of the pain. This suggests that the effect may be non-specific.

ii. In all the fourteen cases the patients' leprosy condition deteriorated clinically and bacteriologically while on toxoid.

The original claims for this remedy have therefore failed to be confirmed and it is not recommended as a method of treatment.

Clinical investigation.

This has been continued throughout the year. Special attention has been given to the so called 'intermediate' or 'border line' case. The view stated last year that the major tuberculoid type does not tend to become lepromatous has not been modified. It appears that the group of cases styled 'border line' or 'intermediate' may also be a true type as the tendency for their transformation into leproma seems so far to be the exception rather than the rule. Out of 26 cases studied during the past four years only two appear to have definitely become leproma. The lepromin test is usually negative, but a few cases have shown a slight positive (one plus sustained up to the third week), during the stage of maximum activity. We have reason to believe that certain cases which may be diagnosed as leproma, but which apparently respond rapidly to treatment, may not be true leproma but should be placed in this group. If this is so, it again emphasises the need for care in assessing the value of any given drug in leprosy.

Child investigation.

The intensive study of the development of lesions in children has been continued, and there are now 440 children from which such a study is possible. Special attention has been given to the development of lesions of leprosy and the study of the lepromin reaction.

Development of lesions—One of the most important investigations undertaken in the children's investigation unit is the study of the progress of lesions of leprosy. Within recent years the theory that a considerable amount of leprosy in children, especially of the neural type, becomes spontaneously arrested has gained ground. Information concerning this matter naturally takes several years to collect. Table VI has been compiled from evidence at the Silver Jubilee Children's Clinic, Saidapet.

Table VI.

Assessment of progress up to the end of October, 1941.

Observation Group.

Type	Total	Total M.	Total F.	Much improved	Improved	Stationary	Worse
Nl Ns. ..	85	55	30	14.1%	29.4%	37.6%	18.8%
Nl Nt Minor ..	101	64	37	20.8%	39.6%	37.6%	2.0%
Nl Nt Major ..	24	18	6	12.5%	62.5%	20.8%	4.2%
Incipient ..	18	8	10	..	27.8%	27.8%	44.4%
Total ..	228	145	83	15.8%	37.3%	35.1%	11.6%

Table VI analyses the results of progress in 228 children in the observation group who have had no treatment. Only 18.8% in the simple neural group, 2% in the minor tuberculoid group and 4.2% in the major tuberculoid group became worse, but 44.4% in the incipient group showed deterioration. Space does not permit of detailed discussion of this subject but it confirms the opinion that probably at least 50% of neural leprosy among children in Saidapet is benign, and emphasises the necessity for caution in any statement with regard to improvement of neural cases under treatment. This question concerning lepromatous cases has not been investigated because in the majority of instances one does not feel justified in withholding treatment. One case of early lepromatous leprosy has become negative without treatment in the Saidapet clinic.

Another matter of importance is whether neural cases tend to become lepromatous or not. Table VII gives an analysis of the percentage of cases which become lepromatous during the past four years.

Table VII.

	Total	No. Lepromatous
Simple Macular (N)	124	7 (5.6%)
Tuberculoid Macular (N) Minor .. .	121	2 (1.7%)
Tuberculoid Macular (N) Major . ..	43	Nil
Incipient	23	5 (21.7%)

This table tends to confirm the opinion already given that the most serious types of lesions in childhood are the simple macular type of neural leprosy and the incipient form of the disease.

Study of lepromin reaction—The total number of lepromin reactions done is 423. Further evidence has accumulated to confirm the opinion expressed that the most important single factor breaking down cellular resistance in leprosy is prolonged contact with an open case and that the lepromin reaction is not significantly influenced by family predisposition.

A comparative study of the reaction resulting from lepromin made at the School of Tropical Medicine, Calcutta, with that made at the Lady Willingdon Leprosy Sanatorium has been done in 86 cases. Tables VIII and IX illustrates this.

Table VIII.

	LADY WILLINGDON LEPROSY SANATORIUM			CALCUTTA		
	No. of cases	No. of positives	Percentage	No. of cases	No. of positives	Percentage
Nl Ns. ..	29	13	44.8	29	6	20.6
Nl Nt Minor and Major	27	17	62.9	27	13	48.1
Nl Incipient ..	9	2	22.2	9	1	11.1
Lepromatous ..	14	..	..	14	..	..
Suspicious and healthy	7	6	85.7	7	6	85.7
Total ..	86	38		86	26	

Table IX.

Comparative tests between Calcutta lepromin and Lady Willingdon Leprosy Sanatorium lepromin in the same case given at the same time.

	Strongly Positive 4+ & 3+	Positive 2+ & 1+	Negative
	Percentage	Percentage	Percentage
Lady Willingdon Leprosy Sanatorium ..	14.0	61.6	24.4
Calcutta ..	10.4	55.9	33.7

STANDARD OF CLASSIFICATION.

3— 5 mm	.. 1 plus
5—10 mm	.. 2 plus
Above 10 mm	.. 3 plus
Ulceration	.. 4 plus

The reactions are stronger with the Lady Willingdon Leprosy Sanatorium lepromin than with that of Calcutta. It was also noted that the primary reaction in the Lady Willingdon Leprosy Sanatorium lepromin

was usually more severe. Further in the Calcutta lepromin there was a distinct period of quiescence before the secondary reaction was noted whereas in the Lady Willingdon Leprosy Sanatorium lepromin the primary reaction tended to merge into the secondary reaction. Lastly the reading of the results of the Calcutta lepromin were easier as the reaction was more clear cut although less severe. The probable explanation of this is that the Calcutta lepromin is a finer preparation and therefore the reactions due to secondary causes such as the presence of tissue material have been largely eliminated. This work tends to confirm the more detailed observation which have been made by the School of Tropical Medicine, Calcutta.

Animal Experiments.

With the cooperation of the King Institute, Guindy, animal experiments have been continued. Inoculation of leprous material intraperitoneally into monkeys has continued to give negative results. Further experiments are being continued in this direction and further studies on the lepromin reaction in animals is being pursued. The findings of these experiments will be reported in due course.

PLAGUE.

24. Plague researches under the Director, Haffkine Institute, Bombay.

FIELD TRIALS OF SULPHATHIAZOLE AND SULPHAPYRIDINE.

During the year H. E. H. the Nizam's Government built a special plague hospital and provided facilities at Latur for treating cases of plague. Opportunities thus offered were utilized for conducting further trials into the value of sulphathiazole and sulphapyridine in the treatment of plague. In all 267 cases of plague were treated; the first 82 cases were treated with sulphathiazole, and thereafter cases were treated in strict rotation with sulphathiazole and sulphapyridine, no selection of cases being made.

On admission 0.5 c.c. of blood of each patient was drawn and plated on two agar slopes to determine the presence or absence of septicaemia and its degree at the time of admission. In the absence of septicaemia, bubo was also punctured and sucked-up material plated.

During the trial special attention was paid to the determination of optimal doses of the sulphonamides used. For this purpose samples of blood were taken every four hours on the first day of the treatment and once every 24 hours thereafter. It was found that a blood concentration of 5 to 9 mg. per 100 c.c. gave the best results. To attain this concentration of sulphathiazole, on an average, 9 g. of the drug on the first day and 7 g. on subsequent days were needed. In the case of sulphapyridine 7 g. on the first day and 4 g. on subsequent days were needed.

From the number of treated cases, 50 cases are excluded — 35 died within 24 hours of admission *i.e.* before the drug could act, 7 left the hospital and 8 for other reasons. For controls the cases treated in the previous three trials with iodine are given here for comparison—the control cases which died within 24 hours of admission are similarly omitted.

All cases of bubonic plague treated.

Drug	Number of cases	Number of deaths	Case mortality per cent
Sulphathiazole ..	147	33	22.4
Sulphapyridine ..	70	21	30.0
Controls—given the usual hospital treatment *i.e.* iodine intravenously	149	80	53.6

Since the presence or absence of septicaemia at the commencement of the treatment is the one important factor which decides the issue, the results of the treatment in septicaemic cases are given separately.

Cases with plague septicaemia at the commencement of treatment.

Drug	Number of cases	Number of deaths	Case mortality per cent
Sulphathiazole ..	62	26	41.9
Sulphapyridine ..	33	19	57.5
Controls—*i.e.* iodine intravenously ..	75	68	90.6

Whether the apparent differences in the case mortalities shown above represents a true difference we are not in a position to say yet, but on the question of toxic symptoms developing from the drugs, the observations were clearly in favour of sulphathiazole.

Plague vaccine.

A. Serological study of strains—For a serological study of the antigenic structure of the plague bacillus and the suitability of strains for the preparation of vaccine and sera, agglutination, cross agglutination, agglutination after absorption and cross-absorption, and precipitation tests were employed. Twelve strains belonging to (*a*) virulent (*b*) protective avirulent, (*c*) non-protective avirulent and (*d*) Pasteurella pseudo-tuberculosis groups were employed and 1,100 sets of tests were conducted. Though the work is not complete yet certain trends are clear.

The agglutination tests show the bacillus to be possessed of two antigenic components—one specific to the plague bacillus and the other common to the Pasteurella group. The test qualitatively differentiated the different groups of organisms used, but did not yield quantitative results to measure antigenic differences between different strains.

The precipitation reaction seems to be much more promising. We were able to prepare the specific antigen in a much purer form than last year by using a protein-free liquid medium. By the use of this specific substance, results were obtained which show that it may be possible to measure quantitatively the antigenic differences between different strains.

B. Study of the effect of various factors involved in the preparation of a vaccine on protective power of the resultant vaccine—The serologically tested strains were employed for the following tests.

(1) *Agar grown and broth grown vaccines*—During the year a method was worked out for counting the number of organisms in vaccines and it was therefore possible to make agar and broth vaccines containing approximately the same number of organisms namely, 1000 million organisms per c.c. Both vaccines were heat killed at 54°C for 15 minutes and carbolised (0.5%).

	Mouse protective dose.
Agar vaccine (average of 8 experiments) ..	0.0065 c.c.
Broth vaccine (average of 9 experiments) ..	0.0060 c.c.

(2) *Method of killing cultures*—Besides heat, merthiolate sulphathiazole and alcohol (Felix) were used for killing cultures.

Agar vaccines	Mouse protective dose.
Heat, 54°C	0.0065 c.c.
Merthiolate, 1 in 500,000	0.0068 c.c.
Sulphathiazole, 1%	0.0070 c.c.

Alcohol (Felix) gave a very poor vaccine.

Broth vaccines	
Heat 54°C	0.006 c.c.
Merthiolate, 1 in 500,000	0.006 c.c.

(3) *Avirulent live vaccines*—Agar vaccines were made from Ottens (Tjs) avirulent strain, Madagaskar (E.V.) strain and Haffkine Institute (53/H) strain. Each vaccine was made to contain 1000 million organisms per c.c.

	Mouse protective dose.
Haffkine Institute (53/H)	0.0005 c.c.
Ottens strain (Tjs)	0.0010 c.c.
Madagaskar (E.V.)	0.0010 c.c.

(4) *Effect of killing avirulent vaccines*—Vaccine used in experiment 3, given above, were also killed by heat and merthiolate.

	Mouse protective dose.
Heat killed	0.0070 c.c.
Merthiolate killed	0.0057 c.c.

Evidently the live avirulent vaccines are the most potent, but there are good reasons against their use. Their avirulence is not absolute, it is merely a high degree of attenuation. If the attenuation goes beyond a certain point the antigenic power of the organisms disappears. Therefore the organisms must be used with a certain residual virulence. Thus there is a risk of their gaining virulence: Keeping qualities of the vaccines appear to be poor. The different methods of killing cultures tested do not show any improvement on heat (54°C). The broth and agar vaccines are equally good, except that it is easier to make broth vaccines in bulk.

Casein hydrolysate medium.

At the Haffkine Institute the routine broth used is made from mutton hydrolised with HCl. Experiments have been carried to prepare broth from casein. They show that casein will yield a broth free from precipitable protein. Incidently it will also be cheap.

Preservation of the virulence of plague cultures.

To overcome the difficulties of maintaining the virulence of plague cultures the method of drying from the frozen state was studied. Of the various substrata used, namely, fat-free milk, inactivated normal horse, rabbit and guineapig sera, various concentrations of gum-acacia and combinations of gum and sera, 5 per cent gum-acacia has so far given consistent result. As high a survival rate as 45 per cent was attained. The virulence is also maintained. The work is being continued to improve the technique.

TUBERCULOSIS.

25. Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta.

TUBERCULOSIS IN RELATION TO INDUSTRY.

Serial Examination of all workers.

The total number of workers of the industry examined and their distribution according to age, sex, religion, nature of work and the province of origin were reported last year. The general incidence of tuberculous infection and tuberculin sensitiveness (82.4%) among the workers in the industry were also reported last year along with their relationship to age, sex, religion, province of origin and section of the industry.

Of the workers (2,100) selected for examination, 784 have been X-rayed during the year, making a total of 1,656 examined with X-ray. It has not been possible to X-ray the remainder because of their leaving the station. Among those who have been X-rayed so far, 112 or 6.8% showed 'definite' and 70 or 4.2% 'probable' radiological evidence of active pulmonary tuberculosis. Thus a total of 11.0% in the selected group or at least 3.7% among the total workers of the Industry showed radiological evidence of active lesions—a high incidence among the polulation examined. Evidence of 'arrested' lesions was detected in 85.4% of the selected group.

Among the X-rayed group, out of 115 cases with suspected clinical signs 48 or 41.7% showed 'definite' and 10 or 8.7% 'probable' evidence of active disease; out of 1,221 high reactors 49 or 4.0% showed 'definite' and 53 or 4.3% 'probable' disease and out of 320 cases with evidence of bronchitis 15 or 4.7% showed 'definite' and 7 or 2.2% 'probable' disease. The incidence of arrested lesions was found to be highest (91.2%) with the last group of cases.

INCIDENCE OF TUBERCULOUS DISEASE ACCORDING TO CERTAIN GENERAL FACTORS.

(*a*) *Age*—The highest percentage (11.3%) of both 'definite' and 'probable' disease was detected in the 50-60 years age group. The percentage of 'arrested' disease, however, was almost equally distributed in the younger age groups (25-50 years).

(*b*) *Sex*—The incidence of active disease detected was higher in females (15.3%) than in males (10.7%). Males, however, showed a higher incidence of 'definite' disease (6.8%) and females of 'probable' disease (11.5%).

(*c*) *Religion*—The incidence of 'definitely' active disease was 7.9% among the Mahomedans and 6.1% among the Hindus, while that of 'probable' disease was exactly equal (4.2%) in the two communities. Hindus showed 86.2% and Mahomedans 83.8% of 'arrested' lesions.

(*d*) *Provinces*—The incidence of active disease, both 'definite' and 'probable' was highest among the workers from Nepal (28.0%=21.8%+6.2%) and lowest among those from Madras (5.2%=2.6%+2.6%). 'Arrested' lesions, however, were highest among the workers from Bengal (89.2%) and lowest among Nepalese workers (71.8%).

(*e*) *Sections of the industry*—The highest incidence of 'definitely' active disease was detected among the Durwans (28.0%), that of 'probably' active

disease among the 'coolies' (8.2%) and that of 'arrested' lesions among the 'Engineering mechanics' (93.1%) of the industry.

Other findings—Out of the 4,816 workers examined 5.6% showed 'Jute dermatitis' and 1.8% nasopharyngeal catarrh'.

Examination of contacts of 'definite' and 'probable' cases detected.

During the current year, 160 contacts, both adults and children, making a total of 500 contacts of the available cases of pulmonary tuberculosis (110 'definite' and 60 'probable') detected, were examined physically and with tuberculin test. Of these contacts 82.6% reacted to tuberculin. Of a total of 165 contacts earmarked for radiological examination, 108 have been X-rayed during the current year making a total of 150. Of these X-rayed 'contacts', 10 or 6.6% showed 'definite' and 7 or 4.6% 'probable' evidence of active disease, or a total of 11.2% active disease in the selected group or an average incidence of 3.4% among the total number of contacts traced out.

Examination of the relatives of the workers.

As reported last year, 1,107 relatives residing in barracks and bustees (in an industrial environment but not employed in the mill) were examined and tested with tuberculin and that 65.2% gave a positive Mantoux test. Out of a total of 240 cases selected for X-ray examination, 181 cases, which were left over, have been X-rayed during the current year. Among these 240 cases, 20 or 8.3% showed 'definite' and 10 or 4.1% 'probable' evidence of active disease, or a total of 12.4% active lesions in the selected group, or an average incidence of 2.7% among the total number of relatives examined (1,107).

Two hundred and fortyeight relatives living in the contiguous villages (non-industrial environment and not employed in the mill) have been examined clinically and by tuberculin test during the current year. This makes a total of 1,100 relatives of this class examined up-to-date. Of these, 744 or 67.6% proved to be tuberculin positive. Of 150 persons earmarked for X-ray examination, it has been possible to X-ray 45 during the current year, making a total of 65 cases X-rayed. Among these, 5 showed 'definite' evidence and 2 'probable' evidence of active lesions.

There were 147 X-ray 'definite' and 87 'probable' cases of pulmonary tuberculosis. Among 159 cases thus far examined during the current year (111 'definite' and 48 'probable'), acid-fast bacilli have been detected in 45 persons of the 'definite' group and in 2 persons of the 'probable' group.

Survey of the dwellings of the workers.

Fortyfour dwelling houses in the adjoining villages have been surveyed during the current year, making a total of 224 houses in the villages surveyed up-to-date. The results of these findings, as well as of 1,522 dwellings in barracks and bustees, are being analysed.

Follow-up of tuberculin negative cases and X-ray diagonsed tuberculosis cases.

Of a total of 846 mill workers who failed to react to tuberculin (1/1000 dilution of O. T.) previously, so far 612 have been retested with tuberculin

(1/1000 and 1/100 O.T.) after a period of about 3 years. Five hundred and thirtythree among them have been found to be tuberculin positive. Eighty-four of these persons have given strong reactions of 3+ and 5 persons 4+ category. The strong reactors are to be X-rayed. Further follow-up work is being carried on.

Fortyeight persons who previously gave evidence of X-ray 'definite' and 30 with 'probable' lesions have been clinically reexamined. Further follow-up work is being carried on.

Contrast surveys.

In order to arrive at useful and valid conclusions regarding the influence of industry on tuberculosis in India, a contrast survey is being carried out among non-industrial village population of approximately the same social status as that of the mill population.

During the current year, 1,200 individuals from 200 homes in non-industrial villages situated about 5-7 miles away from the Angus Jute Works have been examined and their homes surveyed. Among these, 511 or 42.5% proved to be tuberculin positive. Of the 91 cases selected for X-ray examination, 25 have so far been X-rayed, of whom 5 showed 'definite' evidence of active disease. Of these 5 'definite' cases, 3 have shown acid-fast-bacilli in their sputum.

Tuberculosis among 'home contacts' of known phthisis cases.

During the year, 113 contact children of 15 years and under have been examined making a total of 1,104 children from 473 tubercular homes to-date. The distribution of the cases has been as follows:—

(*a*) Age—0-2 yrs., 152; 2-5 yrs., 295; 5-10 yrs., 439; 10-15 yrs., 218.

(*b*) Sex—Males—626; females—478.

(*c*) Religion—Hindus—711; Mahomedans—208; children—150 and others—35.

(*d*) Nature of contact:—

(1) Sources:—Positive sputum—809; negative sputum—129 and unknown sputum—166

(2) Closeness of contact—Intimate—997; Distant—107

(3) Continuity of contact—Immediate—792; Recent—262 and Remote—50

(*e*) Nature of Examinations done—

Mantoux test done on 990 cases.

Radiological examination done on 860 cases.

Both the above examinations done on —746 cases.

The total number of positive reactors was 642 (64.9%). The average incidence of demonstrable X-ray lesion was found in 41.1% of whom 10.4% showed 'active' parenchymal lesions, 5.0% 'arrested' lesions and 25.7% tracheobronchial adenopathy.

Findings of tuberculous infection and disease in relation to some important factors.

(*a*) Age.

Age group.	No. Examined	Tuberculin reactors	Active lesion.	Arrested lesion.	Tracheobronchial adenopathy	Total demonstrable X-ray evidence of disease.
—2 yr.	152	48.2%	11.9%	2.7%	29.3%	43.9%
2+ yr.	295	62.6%	11.1%	2.5%	33.0%	46.6%
5+ yr.	439	69.2%	10.9%	6.0%	22.3%	39.0%
10+ yr.	218	71.2%	8.0%	7.5%	20.2%	33.7%

The rate infection, thus, varies *directly*, while that of demonstrable X-ray evidence of disease *inversely*, with age. The incidence of 'active' parenchymal decreases with age, very slightly in the earlier age periods. The 'arrested' lesion, however, increases with age while the tracheobronchial adenopathy at first increases and then decreases with age.

(*b*) Sex and community.

As before the rate of infection and disease was found to vary slightly between the different sexes and communities.

(*c*) Contact.

(1) Source: The incidence of both infection and disease was highest in the 'open' sputum group (infection rate 66.7% and disease rate 44.2%) and lowest in the 'negative' sputum group (infection rate 55.4% and disease rate 22.5%).

(2) Closeness of contact—As before the intimate contact shows a higher percentage of reactors (66.8%) than the distant contacts (46.4%). The incidence of 'Active' parenchymal lesion was higher among 'Intimate' contacts (10.9%) while that of 'arrested' lesion and tracheobronchial adenopathy was higher among the 'distant' contacts.

(3) Continuity of contact—The incidence of infection and both active and 'arrested' parenchymal disease was highest in the 'recent' contact group. The tracheobronchial adenopathy was, however, highest in the 'remote' contacts.

Home Survey—Of the 473 tubercular homes accommodating these 1,104 children, 112 homes in Calcutta containing 359 children have so far been surveyed.

TYPING OF TUBERCLE BACILLI FROM PATHOLOGICAL MATERIALS BOTH PULMONARY AND NON-PULMONARY.

Twentytwo strains of tubercle bacilli from sputum have been isolated during the year, making a total of 250 strains, of which 123 are from non-pulmonary sources, 78 from pleuro-pulmonary sources and 49 from sputum.

All the strains of the former two groups and 30 strains of the last group have proved to belong to the *human* type. The remaining strains are under investigation. The sources of the strains have been as follows:

(1) Non-pulmonary sources—Cervical—67; Axillary—7; Inguinal—1; Mesenteric—12; Tonsil—2; Cold Abscess—21; Skin—5; Urine—4; Cerebro spinal—1; Stomach wash—1; Stool—1; Pericardial—1.

(2) Pleuro-pulmonary sources—Pleural fluid—36; Tracheobronchial gland——38; Lung tissue—4.

(3) Sputum—(Sputum from both diagonsed and undiagnosed cases of pulmonary tuberculosis—49).

26. Enquiry into the incidence of positive Mantoux reactions and latent tuberculosis as judged by skiagrams in medical students and nurses under Major G. F. Taylor, I.M.S. at the K. E. Medical College, Lahore.

The following results were obtained during 1940-41:—

MANTOUX REACTIONS.

	Total	Mantoux positive.	Mantoux negative.
1st year students ..	73	50 or 68.5%	23 or 31.5%
2nd year students ..	81	52 or 64%	29 or 36%
3rd year students ..	54	40 or 74%	44 or 26%
4th year students ..	57	48 or 84%	9 or 16%
5th year students ..	57	49 or 86%	8 or 14%
	322	239 or 74.2%	83 or 25.8%

There is a higher incidence than in previous years of positive reactors in the first year students.

During the period 32 students who had previously been negative became positive reactors. As in previous years, these 32 students have been examined clinically, and radiologically and have shown no clinical signs of tuberculosis apart from the positive Mantoux reaction.

Two students who had been positive reactors became negative during the year, a change which is not at present understood.

Fortynine students showed very severe reactions with 1 c.c. 1/1000 tuberculin solution.

X-RAY EXAMINATION.

As in previous years, X-ray photographs were taken of the chest of the new first year students, of all latent cases from previous years, and students whose Mantoux reaction had changed from negative to positive.

None of the students who had changed from negative to positive showed radiological evidence of lung tuberculosis.

The following table shows the result of skiagrams which have been taken during the period of this work :—

Year.	Total X-Rays.	Positive X-ray cases with toxaemia etc.	Positive X-ray cases without toxaemia.	Total X-ray positive cases.	Doubtful X-ray cases.
1st year 1938-39 ..	86	2 = 2.3%	8 = 9.3%	11 =12.8%	3 = 3.5%
1st year 1939-40 ..	99	1 = 1.1%	8 = 8.9%	9 = 1.0%	4 = 4.4%
1st year 1940-41 ..	86	3 = 3.5%	4 = 4.0%	7 = 8.1%	1 = 1.2%

The students with positive X-Rays of the chest and with signs of toxaemia have been advised treatment in sanatoria and elsewhere. The other students are being kept under observation.

Summary and proposals.

From these results it is obvious there is a high rate of latent tuberculosis in medical students at the time they begin their medical work. As these students come from the same classes as officers and others, recruited in the Army, it is suggested that the I. R. F. A. should take up with the Army authorities the need for miniature radiography of all recruits to the Army.

27. Epidemiological enquiry on tuberculosis at Saidapet under Dr. R. G. Cochrane, Chief Medical Officer, Lady Willingdon Leprosy Sanatorium, Chingleput, Madras.

The enquiry was continued throughout the year and in this report the following points are considered :—

i. Fresh survey area
ii. Resurvey area
iii. Change in the Mantoux reaction
iv. Movement of high reactors
v. Morbidity and mortality
vi. Study of the degree of positive reaction of the Mantoux test in relation to contact.
vii. Follow-up work
viii. Future programme.

Fresh survey area.

During the period under review in the fresh survey area 1,902 tests were made. The area chosen represents 12 streets and 2 institutions with residential hostels attached and a small group of persons living in isolated places. The number of adults who had the test is 987. Of these 193 or 19.5% did not react to the test. Seven hundred and ninetyfour or 80.5% showed a positive reaction.

Nine hundred and fifteen children had the test, and of them 581 or 63.5% showed a negative reaction, and 334 or 36.5% showed a positive reaction. Considered as a whole in the total of 1,902 tests made in this area, 774 or 40.7% showed a negative reaction, and the remaining 1,128 or 59.3% showed a positive reaction. These findings more or less agree with the findings of the 1938-39 survey.

As reported last year, so this year the dilution of tuberculin used was 1 : 500 ; this was recommended because it avoids the unpleasant consequences which occasionally arise when a 1 : 100 dilution is used.

Resurvey area.

In the resurvey area the total number of tests done during the period was 657. Of these 238 or 36.2% did not react to the test. Four hundred and nineteen or 63.8% showed a positive reaction. Of these 317 were adults of whom 53 or 16.7% showed a negative reaction, and 264 or 83.3% showed a positive reaction. In 340 children 185 or 54.4% were negatives and 155 or 45.6% showed a positive reaction. In the same area the findings of the last survey showed 59% positives in the general population. Among adults alone it was 70.7% and in children it was 42%. It is interesting to note that while there has been a slight increase in the general rate and a greater increase in the adult rate the child rate continues to be nearly the same.

Change in the Mantoux reaction.

Two hundred and ninetyfive persons who had the Mantoux test in 1938-39 survey were retested during this period making a total of 542 retested up-to-date. Of these 180 have increased their reactions, 83 have decreased reactions and in 279 their reactions are unchanged. Of the 542 persons tested 268 were adults and in them 106 have increased reactions ; 36 have their reactions decreased ; and 126 had their reactions stationary. Two hundred and seventyfour were children and in them 74 showed increased reactions, 47 decreased reactions, and 153 had their reactions stationary.

Movement of high reactors.

Out of a total of 117 high reactors who were not living in Saidapet at the time of 1938-39 survey it was found that 66 have come in from urban areas, 44 from rural areas and 7 from semi-rural areas. It is now known that of high reactors detected in 1938-39, it is possible to account for the movement out, of 122 high reactors. Seventyone moved away to urban, 18 to semi-rural and 33 to rural areas. Six of the high reactors of the 1938-39 survey have since died. The Municipal register has not recorded any of these deaths as due to tuberculosis. The number of high reactors whose movement is definitely known is about the same for movement in, and movement out.

Morbidity and mortality.

(*a*) *Morbidity*—Persons taken up for the study of morbidity belong to four groups : (1) all persons who showed a reaction of 1.5 cms and more to the Mantoux test ; (2) persons who showed symptoms suggestive of tuberculous infection, (3) all contacts of known cases of tuberculous individuals and (4) all children below the age of two who showed a positive reaction. An attempt

was made to get as many of the above class of persons screened by the fluroscope. Physical examination was done for those who on screening were found to have definite or suspicious X-Ray signs of infection, as also for those who had symptoms. During this period 470 persons were screened.

Routine direct sputum smear examinations were done in persons who could bring up any sputum and who gave the samples in receptacles provided by the survey unit. Sixteen cases were found to be positive. Blood sedimentation and leucocyte counts were also included in the determination of the tuberculous state of individuals. During this period 34 active progressive cases, 21 active regressive cases, 3 cases of abdominal tuberculosis and glandular disease in 9 persons were found.

Close contact with persons under observation was maintained through visits made to the homes by the Health Visitor and of visits made by the individuals to the Sunday Clinics held in the Silver Jubilee Children's Clinic. A total of 744 visits was thus recorded.

(*b*) *Mortality*—During the period under review 32 persons died of tuberculosis in the survey area. Twentyfive of these had no adequate treatment. Five were treated in special institutions as out patients. Only one was admitted into a regular tuberculosis hospital. No information was available in respect of one individual. The first information regarding deaths is obtained from the Municipal Health Officer whose staff fill in the individual forms supplied by the survey unit in every case of death which is not obviously due to an accident or is authoritatively known as due to a definite illness other than tuberculosis. The survey unit proceeds to the houses where suspected death from tuberculosis has taken place and verifies the information.

Age grouping and mortality from tuberculosis—From the figures supplied of tuberculosis deaths by the Municipality for the past 5 years it is now found that the largest number of deaths occurred in the age group, 21 to 30 years.

The figures for tuberculous mortality in relation to total deaths in the town area has been worked out. In 1936, the number of deaths from tuberculosis was 30 out of 450 deaths from all causes working out at 6.7%, in 1937, 34 out of 402 deaths or 8.5% ; in 1938, 34 deaths out of 463 or 7.3% ; in 1939, 46 out of 620 deaths or 7.4% ; and in 1940, 36 out of 484 deaths or 7.4%. The above figures were supplied by the municipal authorities and the survey unit is not responsible for the correctness of the same.

Study of the degree of positive of the Mantoux test in relation to contact.

The following tables give a comparison of the Mantoux test between those who have familial contact with an open case and the rate in the general population as a whole.

(1) Mantoux reaction of familial contacts of *open cases.*

	No.	Negative	One plus	Two plus	Three plus	Four plus	No. of positives and percentage.
Adults ..	55	5 9.09%	22 40.00%	11 20.00%	10 18.18%	7 12.73%	50 pr 90.91%
Children ..	35	6 17.14%	7 20.00%	5 14.29%	13 37.14%	4 11.43%	29 or 82.86%
Total ..	90	11 12.22%	29 32.22%	16 17.78%	23 25.56%	11 12.22%	79 87.78%

(2) Mantoux reactions of general population of both the areas combined.

	No.	Negative	One plus	Two plus	Three plus	Four plus	No. of positives and percentage.
Adults ..	2910	628 21.58%	1467 50.41%	307 10.55%	355 12.20%	153 5.26%	2282 78.42%
Children	2631	1626 61.81%	590 22.42%	141 5.36%	223 8.47%	51 1.94%	1005 38.19%
Total ..	5541	2254 40.68%	2057 37.12%	448 8.09%	578 10.42%	204 3.68%	3287 59.32%

The increase in the Mantoux in familial contacts is seen in all degrees of positivity and in a further analysis of the 90 familial contacts—the negatives were strikingly low (12.22%) and the total positive rate high (87.78%). The high reactors, plus two and plus three, of the contact group are more than twice those in the corresponding group in the general rate. The plus fours are three times greater in the contact group. The adult rate is also high for the total positives. The greatest difference being in the plus four group, where the contact rate is more than twice the rate in the general population. The negative rate in children of the contact group is 17.14% as against 61.81% negatives in children of the general population group. More striking is the total positivity rate of 82.86% for the contact group in children as against 38.19% positives for the general population. In the child group whereas the plus one positives for the general population is 22.42%, in the contact group it is 20.00%. In the other three groups however the plus two, plus three and plus four, the rate for contact children is very much higher than for the corresponding groups in children of the general population. The most striking is the plus fours of the children of the contact group who number as much as six times more than the general population. The number of familial contacts so far studied is only 90 and therefore definite deductions cannot be made from this small group. More data are being collected so as to include material from previous survey figures.

Follow-up work.

The 1938-39 survey party suggested 3 groups of cases for follow-up work. They were, (1) persons with active disease needing treatment, (2) persons with suggestive signs of active disease who needed further observation before it could be decided whether the treatment is necessary and (3) those who showed signs of disease probably not active but should be checked up from time to time. Emmigration, failure to co-operate or death limited this number to 83. Follow-up charts were recorded for all persons in these groups. The present state of health is assessed on Mantoux test, fluroscopic screening, physical examination, record of weight, examination of sputum, blood examination and symptomatology. An attempt to include as many of the above units of assessment is made in every case. It was found that 46 individuals are in fairly good health and have little or no complaints to make. Twentytwo are in stationary health with occasional spells of illness. Seven have definitely gone worse. Detailed investigation has proved that seven of the referred cases are non tuberculous. Only two persons who had definite tuberculous disease in 1938-30 referred for attention had proper treatment in an institution.

Home isolation—The possibility of home isolation was investigated in 16 cases of tuberculosis in the survey area during the period under review. The possibility of home isolation is judged on the following points: (1) adequacy of room, floor and cubic space and ventilation, (2) the use of the room exclusively by the tuberculous individual and by others, (3) economic status for isolation judged on the possibility of persons being able to own or hire a single room for himself or herself, (4) whether the person has to maintain himself and his family, (5) general level of intelligence and education, (6) presence or the absence of the practice of indiscriminate spitting and how far it is controlled by the individual and (7) whether the patient had any regular and effective treatment. On these points it was found that isolation was possible and practised in only one case, and in two cases it was possible but was not practised, and in 13 cases isolation was not possible.

Future programme.

There is evidence that the Manfoux reaction is not necessarily constant in the same individual and therefore a study of the variation of the test is an interesting outcome of this work and will be pursued. The factors causing the change in the Mantoux test will be investigated.

The 1938-39 survey noted some obvious cases of tuberculosis which required immediate treatment. It also gave a list of persons with tuberculosis infection who should be observed and followed up. A study of this group gives some indication of the disease as a community problem. This survey affords another group of individuals whose reactions to the infection may be further studied.

Infection in children—Discussing the 1938-39 survey figures Dr. Benjamin is of opinion that 41.2% infection of children below 15 years is very high. He also observed that this indicated a serious situation with early infection. He infers that infection takes place mainly before the children are in contact with the outside world. The findings in regard to childhood infection as a result of the present survey are generally in agreement with the above opinion. There is a large group of children whose state of infection has now been noted.

A further intensive study will be made of infection in children. Dr. Benjamin has emphasised the importance of this in view of the fact that 41.2%

of the infection occurs in children below 15 years. In this connection there is probably no place more suitable for such a study than Saidapet as this investigation has much in common with the investigation of leprosy in childhood. This last investigation has been under way for five years and the experience gained in that connection will be of great value in any work on childhood tuberculosis. There is thus an assurance that valuable information concerning childhood tuberculosis will be obtained. This disease is mainly one affecting the younger age groups and a similar study as far as we are aware has not been undertaken in India.

Rural areas—It is proposed to select one or two rural areas within reach of Madras for comparison and in this way a gradual picture of rural as well as urban tuberculosis will be built up, possibly further knowledge will be acquired with regard to the spread of tuberculosis from the towns to the villages.

Acknowledgements—Grateful acknowledgements are made to Dr. P. V. Benjamin and Dr. Vasudeva Rao for their advice and help readily given throughout the year. Dr. Sivakrishnan has throughout the year made available his model X-Ray Unit.

28. Enquiry into the prevalence of bovine tuberculosis in Vizagapatam under Dr. N. G. P. Pandalai, Professor of Bacteriology, Medical College, Vizagapatam.

During the year 70 specimens of suspected tuberculous material from non-pulmonary cases were investigated; of these 14 were from glands, 10 cold abscess, 7 ascitis, 11 abdominal tuberculosis (motion and resected gut), 7 meningitis, 5 bone and joint infection, 9 renal infection, 4 middle ear disease, 3 post mortem cases, all clinically diagnosed as tuberculous in origin. These materials were collected from the King George Hospital and other local hospitals and from autopsies done in this institution.

For cultural isolation of the organism Lowenstein-Jensen medium also was employed in addition to Dorset's medium, potato medium, inspissated serum, agar and broth. By these methods fourteen strains were obtained from the materials investigated.

Parallel with cultural methods, guinea-pigs also were inoculated with these materials for isolating the organism. One more strain was obtained by this procedure.

Cultural methods.

The eugonic nature of the growth of the human as opposed to the dysgonic nature of the bovine type, the decided influence of glycerine on the growth of the human type, the difference in the type of growth between the human and the bovine varieties on the same medium and the frequent pigmentary activity of the human variety, were the main criteria relied upon for differentiating the type culturally. Different media including Lowenstein-Jensen's were employed for the study of these characters. In inoculating into media care was taken to transfer identical amounts during each transplantation. Inoculations were done from the first subcultures. The labelled and sealed tubes were then incubated at 37°c. Periodical examinations were made recording the observation. No attempt was made to decide the type under 12 weeks. By this method 12 strains definitely corresponded to human type.

Biological methods.

Two young rabbits were chosen for each strain. Into each rabbit 10 mg. of accurately weighed dry culture were injected intramuscularly. Of the twelve strains that conformed to the human type culturally, strains R37 and R38 caused generalised tuberculosis in the test animals and killed them, the former after 53 and 58 and the latter after 85 and 93 days. As this threw some doubt on the cultural typing, fresh cultures of the strains are being prepared for subjecting them to further investigation.

During the year 180 specimens of cow's milk were examined for the presence of Myco. tuberculosis by the Cowan and Madocks method. None showed the presence of tubercle bacillus nor the typical cell grouping associated with tuberculous infection of the udder.

Both the abatoir and the local veterinary hospital are being regularly visited to obtain cases of tuberculosis in the bovines. So far none has come to notice.

An attempt was made to find out the inhibitory effect of snake venom, prontosil rubrum and copper sulphate on the growth of Myco. tuberculosis. Snake venom and prontosil rubrum did not have any inhibitory effect on five strains subjected to the test in concentrations of 1/1000, 1/500 and 1/100. Copper sulphate in a concentration of 1/8000 showed inhibition of growth in the case of ten strains that were tested.

INDIGENOUS DRUGS.

29. Indigenous drugs enquiry under Bt.-Col. Sir Ram Nath Chopra, I.M.S. (Retd.) at the School of Tropical Medicine, Calcutta.

The study of the following problems has almost been completed and will shortly be communicated:—(1) hypnotic effects of Rauwolfia alkaloids, (2) pharmacological action of stilbene, (3) cataphoretic velocity of red blood corpuscles and the influence of their environment, (4) role of the reticulo-endothelial system in explaining drug action in certain tropical diseases, and (5) sodium and diethyl salts of p.aminophenyl stibinic acid.

INDIGENOUS DRUGS.

(I) *Medicinal plants*—During the present year investigations on a number of indigenous drugs have been completed. (1) *Rauwolfia serpentina*. An easy and suitable method for the isolation of its alkaloids has been worked out. Detailed pharmacological studies on the various alkaloids of the plant have enabled us to identify its pressure-reducing principle and localise the fraction responsible for the sedative and hypnotic effects. From these studies it appeared that the Dehra Dun sample was more suitable in the treatment of hypertension than others. A method for the quantitative assay of this alkaloid is under investigation. (2) Pharmacological investigations on Cerberin, a glucoside from *Cerbera odollam*, have given demonstrative proof of good cardiotonic action. It will be interesting to detail this preliminary study and explore if it could be a substitute for the digitalis group of drugs. (3) *Balsamodendron mukul* has been chemically examined. A comparison of its constituents with those of balsam tolu placed it as a poor substitute for the latter in antiseptic and expectorant properties. In the former benzoic and cinnamic acids and their esters are entirely absent. (4) Examination of the widely distributed tannin bearing plants for the preparation of a concentrated solution of tannic acid for therapeutic use has been undertaken. *Terminalia chebula, Acacia arabica, Acacia catechu* and *Ceriops roxburghiana* are very suitable for the purpose as they contain fairly high percentage of tannins. Tannin solutions prepared from them are being tested in the Bacteriological Department. (5) Several samples of *daturas*—both the white and black varieties—were examined. The well-matured fruits with seeds were found to contain from 0.24 to 0.36 per cent of alkaloid calculated as hyoscyamin. The leaves and roots could possibly be used for liniments and plasters in place of belladonna. An attempt is also being made for converting hyoscyamine into atropine. (6) The cathartic property of myrobalan was not marked in animal experiments. Investigations on different species of cassias have been undertaken. (7) Four specimens of rhubarb (*Rheum emodi*) collected by the Political Agent for Sikkim were chemically examined and compared with genuine samples of English and Chinese rhubarb. Only one sample was found to be up to the B. P. standard. (8) Pyrethrin content of several samples of pyrethrum grown in different parts of India varied from 0.4 to 1.3 per cent. The results have been communicated to the Journal of American Pharmaceutical Association. The pharmacological action of this drug is under investigation at present. It appears that cultivation of this plant in India will be very beneficial and profitable. (9) Essential oils obtained from the following plants have been studied chiefly with regard to their R. W. coefficient values: (*a*) *Ocimum sanctum*, (*b*) *Ocimum basilicum*, (*c*) *Curcuma aromatica*, (*d*) *Pistacia integerrima*, (*e*) *Homalomena aromatica*, (*f*) *Hemidesmus*

indicus. Of these only ocimum species have been found to possess appreciable antiseptic properties.

(II) *Herbarium of medicinal plants*—This work has further progressed during the year.

(III) *Poisonous plants of India*—The first volume of this monograph will soon be published this year and the second volume will soon follow.

Chemotherapeutic investigations.

(1) It has often been suggested that certain well known drugs such as quinine, organo-metallic arsenic and antimony compounds act through the reticulo-endothelial system. The exact mechanism of action of these important drugs is not definitely known. With a view to elucidating this problem efforts have been made to study the action of some of these drugs after blocking the reticulo-endothelial system both in normal and infected animals. Results so far obtained show that the study merits further generalisation. (2) Sulphaguanidine, a drug which has been used with excellent results in bacillary dysentery, was tried in a large series of cases of cholera. Although adequate doses as recently worked out were not given, the mortality rate was definitely decreased. Further trials with proper doses will be carried out in the next epidemic. Sulphaguanidine was also tried in a small series of cases of amoebic dysentery, but appeared to have no curative results. (3) Two newly synthesised antimony compounds—sodium and diethyl salts of p-aminophenyl stibinic acid—are being pharmacologically studied. Preliminary investigations have shown that the sodium salt is definitely less toxic than many other available salts in the market. (4) Pharmacological studies are also being conducted on stilbene, a drug used in kala-azar.

Drug addiction.

Studies have been continued in the domain of the abuse of *Cannabis sativa*, alchohol and eucodal in India. These have recently been communicated for publication.

Substitutes for foreign pharmacopoeial drugs.

The attention of this enquiry is now chiefly directed towards finding out suitable substitutes of pharmacopoeial drugs which are not indigenous to India or are difficult to secure on account of war conditions. We have prepared a list of drugs and their substitutes which closely resemble the B. P. drugs either chemically or in their therapeutic properties. These are being tested chemically and pharmacologically with a view to their wider and more general utilization:

B. P. drugs.	*Indigenous substitutes.*
Gentiana lutea Linn.	*G. chiravita, G. dahurica, G. kurro, Enicostema littorale, Clerodendron inerme, C. infortuatum.*
Polygala senega	*P. telephoides, P. chinensis, P. chrotalarioides.*
Ergot	*Aristolochis bracteata, A. indica, Nigella sativa.*
Rheum palmatum	*R. emodi, R. webbianum.*
Rhamnus purshiana (cascara)	*R. wightii, R. persicus, R. dahuricus, R. purpurius, R. triqueter, Cassia.*

Jateorhiza palmata (calumba)	*Coscinium fenestratum.*
Cephaelis ipecacuanha	*Naregamia alta, Asclepias curassavica, Calotropis gigantea, Randia dumetorum.*
Picraena excelsa	*Ailanthus excelsa.*
Euonymus atropurpureus	*E. crenulatus, E. pendulus, E. tingens.*
Santonin	*Alanta lactone,* active principle of *Inula helenium.*
Salix sps.	*S. caprea, S. acmophylla, S. babylonica, S. daphnoides, S. tetrasperma,* etc.

This is only a preliminary list and a more detailed list is being prepared.

30. Indigenous drugs enquiry under Dr. Khem Singh Grewal at the K. E. Medical College, Lahore.

Hiran tutia.

The work on Hiran tutia was continued. The literature on indigenous drugs was consulted. No references have been found except two only. These do not seem to refer to the drug under investigation. In Watt's Dictionary of Economic Products of India it is mentioned that "Probably root of Colchicum luteum is Haran-tutiya". Colonel Chopra in his book—Indigenous Drugs of India—on page 126 says "Hiranya-tutiya or Haran-tutiya, a medicine of great repute in Afghanistan and Northern India, is a dark brown extract prepared from the aqueous extract of Colchicum luteum and other species".

The colour reactions of the alkaloid isolated in this laboratory do not resemble those of Colchicine. The toxicities of the dry watery extract and the dry alcoholic extract were tested. The powdered drug is more toxic than the extracts from equivalent amount of the drug. Heat seems to destroy the activity of the drug. Further work is being done.

Bhawar. (seeds of cassia tora).

The seeds are used in skin diseases in the Punjab with some success. Ten gms. of the powdered seeds on successive extractions with the solvents yielded extracts in the following proportions:—

Petroleum ether 8.0 per cent; ether 0.6 per cent; ehtyl acetate 1.6 per cent; chloroform 0.4 per cent and alcohol 19.84 per cent. Six pounds of the drug were extracted with hot alcohol. The extract was concentrated under reduced pressure. The concentrated extract was treated with water. The water soluble fraction was studied by steam distillation for any volatile substance; the non-volatile fraction was extracted with ether and amyl alcohol successively.

On steam distillation a small quantity of volatile oil was found. The ether soluble substance was separated into three fractions by treatment with ammonium carbonate, sodium carbonate and potassium hydroxide. Deep orange needles with melting point of 250°-252°C crystallized from dilute acetic acid containing a little pyridine from ammonium carbonate

fraction. Acetyl derivative of the crystallized substance was prepared. This identified it with emodine.

The other two fractions yielded no crystalline substances but the impure substances gave tests of free emodines. The amyl acetate fraction gave esters and glucosides of emodines but could not be obtained in crystalline form. Further work is in progress.

APPRAJIT.

It is used as a purgative. The powdered seeds were successively extracted with the following solvents :—

Benzine extract 11.83 per cent ; alcohol extract 8.3 per cent ; water extract 8.9 per cent ; the preliminary tests showed the drug to contain a fixed oil and a glucoside. Work is in progress.

RATTANJOT.

A very valuable dye of the amino-hydroxyanthra quinone group has been extracted. This has been established by colour reactions and spectroscopic examination of the dye. Its dyeing properties have been studied ; its commercial use as a colouring agent for vegetable ghee is under investigation ; the toxicity of the dye before and after heating is being determined.

CHAKSU.

The pharmacological investigation of Chaksu is being carried out. The actions of its alkaloid have been studied on isolated organs—intestine, uterus, heart, (both frogs and rabbits). Its toxicity in mice, rabbits, dogs and frogs has been worked out. Its effect on heat regulation is being studied and also its action as bacteriostatic against streptococci is under investigation.

PRELIMINARY EXAMINATIONS.

The preliminary examination of the following drugs has been done :—

(1) Amleka, (2) Amalbed, (3) Lana.

Amleka and *Amalbed*—Both these drugs contain oxalates, both soluble and insoluble and free oxalic acid in fair quantity.

Lana—It contains soluble tartrates in large quantity and also contains Vitamin C and Vitamin B_1. Further work is in progress.

31. Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay.

A jelly-like change in the tissues on perfusion of the heart and blood vessels with the solution of the glucoalkaloid from *Solanum Xanthocarpum* has been reported last year. Somewhat similar results were obtained on perfusion with certain known diuretics, such as theobromine, salyrgan and novasurol. Perfusion of the hearts in situ of frogs previously treated with calcium gluconate or with antipyrine was also carried out. In the case of those previously treated with calcium, there was very little "jelly-formation", and the heart continued to beat longer, showing a systolic tendency, than those of the frogs treated with antipyrine which behaved in this respect like the untreated controls.

them had enlarged spleens; 10 cases had infection both with Ascaris and Ankylostoma, 10 had Ankylostoma only, and one had Ascaris only.

There were only 5 cases of microcytic type of anaemia. All these cases had total R. B. Cs. count above 2 millions. Four of these cases were microcytic hypochromic and one microcytic normochronic. The last case turned out to be fatal. She miscarried one week after admission and died the next day. Of the four hypochromic type one took regular treatment and her count and haemoglobin came to normal after 4 months. The second one, though she had no regular treatment at the hospital, was 6 months later traced out and found to be normal. The remaining two could not be traced.

With a view to know if the same type of microcytic anaemia which is found in pregnant women is also common in non-pregnant women and men, as many of these cases as could be found were examined. The procedure employed for examination was the same as for pregnant women.

Non-pregnant anaemic women.

During the year 25 cases were done. In these 25 cases, there were five cases of macrocytic type, one bordering on that type, five microcytic hyphochromic and one simple microcytic type.

One of the five macrocytic type of cases was removed home from the hospital in bad condition. She was reported to have died soon after. The other case which died, was on the border line of macrocytosis. She got worse and was removed from the hospital in a dying condition 22 days after admission. All the other cases are presumed to be all right. They were treated on the same lines as pregnant cases.

Anaemic men.

Twentynine cases of anaemic men were examined. Amongst these 29 cases there were 12 cases of macrocytic type of anaemia, 3 cases were bording on this type and 5 of microcytic hypochromic type. Here again one which was on border line died 15 days after admission. Most of the remaining cases of both these types which could be kept under observation were either largely improved or cured.

Normal cases.

Bloods of 55 cases of men, women and children were examined to find out the general blood condition of this population. Most of these cases were from neighbouring villages. They were mostly Hindus. Distribution according to total R. B. Cs count was as follows:—

Total R. B. Cs. count.				Cases.
Between	2 and 2.5	million per	1 c.mm.	3
,,	2.5 to 3	,,	,,	6
,,	3 to 3.5	,,	,,	4
,,	3.5 to 4	,,	,,	14
,,	4 to 4.5	,,	,,	10
,,	4.5 to 5	,,	,,	10
Above	5	,,	,,	8
				55

This result shows that 18 cases out of 55 may be considered to have normal standard as compared to other standards. Most of the cases fall in the group between 3 and 4.5 millions. What were taken as normal cases were those who were not actually ill at the time of examination. They were doing their normal duties. But in common with rest of the population they cannot be considered as quite healthy. As the idea of this examination was to arrive at the blood standard of the general population, cases as they came were taken up and no attempt was made to select only those who were in perfect health. That they were living under unhealthy condition of life specially as regards malaria is quite evident from the fact that 17 of these 55 had enlarged spleens.

In order to gauge the incidence of anaemia in pregnant women, a census of all pregnant women in the town of Sirsi was taken in the month of July and August. One hundred and seventyfive cases were seen. They were again seen second time in the month of September and third time in November. During this period of 5 months many of them had of course delivered. On the third round 116 were still carrying. To avoid unpopularity no attempt was made to examine bloods of all these cases, though some of them did come to the dispensary and were taken up for examination. Amongst these women there were a few cases which looked anaemic but only one case turned out to be macrocytic type of anaemia and ultimately proved fatal.

Amongst the 26 cases of macrocytic type of anaemia in pregnant women which were recorded during the year there were only four cases which came from the Sirsi town. All the rest were from neighbouring villages.

Besides the blood examinations, stools of almost all cases except the normals were examined for ova. Out of a total of 123 stools examined only 15 gave negative results. Sixtyone cases had both Ascaris and Ankylostoma, 12 only Ascaris and 35 only Ankylostoma.

Gastric analysis was done in all in 22 cases. In almost all these cases the free HCl was below normal. There were only 3 cases with no acid at all.

Qualitative Van den Berg reaction was done in the large majority of cases. The results did not materially help in classifying the anaemias.

34. Study of the blood picture in normal pregnancy under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta.

The object of this study is to endeavour to plot the physiological blood picture of the pregnant Indian woman by examination of 100 clinically normal women at each month of pregnancy, from the third month. The study is being undertaken in collaboration with Dr. R. B. Lal, Professor of Vital Statistics and Epidemiology of this Institute and Dr. Subodh Mitra, Honorary Obstetrician to the Chittaranjan Seva Sadan, and the Carmichael Medical College Hospital. Dr. L. E. Napier was consulted with regard to the haemotological technique employed and the Department of Helminthology of the School of Tropical Medicine kindly undertook the detection of ova in specimens of faeces.

The study commenced in September 1939 and 614 cases had been examined before the employment of the staff appointed under the Indian Research Fund Association's grant for the year 1941-42.

Cases are obtained from the pre-natal clinics of the following institutions :—

Institute Maternity and Child Welfare Centre,
Chittaranjan Seva Sadan,

Sishu Mangal Pratisthan, and from among Dr. Subodh Mitra's private patients.

Up to date a total of 1107 first specimens have been taken and in 388 cases biochemical examinations were also made. Of these 1,107, 385 have been temporarily set aside as certain supplementary examinations (for example, examination of faeces for ova) were not available. In a further group of 232, clinical abnormalities were present.

In 220 cases the woman was judged clinically normal, taking into consideration certain laboratory tests also. In regard to pregnancy the distribution of this group is :—

Under 3 months	..	16
3 months +	..	19
4 months +	..	25
5 months +	..	25
6 months +	..	22
7 months +	..	36
8 months +	..	26
9 months +	..	45

In this group biochemical examinations were available for 67 cases : namely, under 3 months, 6 ; at 3 months +, 5 ; at 4 months +, 10 ; at 5 months +, 3.; at 6 months +, 3 ; at 7 months +, 11 ; at 8 months +, 10 ; at 9 months +, 19, respectively by months of pregnancy. One hundred and twenty women had two or more haemotological examinations made : in 56 of these, a postnatal examination taken six weeks after delivery is included.

A further group of 128 cases appeared to be comparable to the above group in that the woman was judged normal on physical examination, but exhibited upon laboratory investigation a slight abnormality, which however did not affect the blood picture. This made a total of 318 cases which may be expected to yield the blood picture of normal pregnancy. With the addition of this group, the figures for total examinations in relationship to months of pregnancy are as follows :—

Under 3 months	..	23
Over 3 months	..	29
4 months +	..	35
5 months +	..	38
6 months +	..	43
7 months +	..	64
8 months +	..	59
9 months +	..	50

The difficulty of obtaining certain drugs and chemicals, under war conditions, has affected adversely the number of biochemical tests undertaken and has made it necessary to change the method of estimating cholesterol in the blood.

As can be seen from the distribution of normal cases according to months of pregnancy, the figures are yet too small from which to draw conclusions. No attempt has, therefore, yet been made to examine the data available, but preliminary tabulation and analysis will be undertaken in the near future.

35. Pregnancy follow up survey under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta.

The object of this study, which was suggested by the Maternal Mortality Enquiry Committee at its meeting in December 1940, was to ascertain to what extent the extraction of data from the records of maternity and child welfare centres in Calcutta would throw light upon the problem of vital loss up to the end of the first year of life.

Pregnancies terminating between 1st January 1939 and 31st December 1940 were chosen for the study and nine centres were invited to submit all prenatal and infant records for these years.

Four thousand nine hundred and fiftyfive pre-natal records were submitted by seven centres. Of these, 2,742 only could be utilised as for the remainder no record of termination of pregnancy was obtainable even after pairing with the infant cards. In view of the factors under investigation, infant cards without pre-natal records of the mother were not utilised.

Following are the numbers of cards submitted by each centre and of those which could be utilised for the enquiry :

Centre No. and Name	Cards supplied.	Cards used.
1. Calcutta Health Week Centre	401	266
2. St. John Ambulance Clinic	139	44
3. Institute of Hygiene Welfare Centre ..	1,030	769
4. Shasthya Niketan	421	408
5. Clive Jute Mill Centre	441	221
6. Kidderpore Centre	1,001	390
7. Entally Centre	1,522	644
	4,955	2,742

Each termination of pregnancy was recorded along with the following data relating to the mother :— Age, age at cohabitation, interval from termination of last pregnancy to the commencement of the present pregnancy, parity, history of previous pregnancies, present economic status and diet, existence of venereal disease, nature and adequacy of pre-natal care, recorded abnormalities or diseases in the pre-natal period, attendant at, and place of delivery, the nature of the labour and of the post-partum period.

In regard to the infant, the following data were extracted : sex, maturity, physical condition, mother's health up to one month after delivery, nature and adequacy of feeding during the first month, adequacy of neo-natal care, contact with other children within one month of birth, length of record of the infant if born alive and age and cause of death if dead within one year.

Only a very superficial analysis of the material is yet available.

Two thousand seven hundred and fortytwo terminations of pregnancy were recorded, including 30 pairs of twins. Single live-births number 2,537 (2,318 full term and 219 premature), still-births number 85 (50 full term and 35 premature), abortions 71, macerated foetus 18 and monster 1.

Action of the drug was studied also on mammalian hearts by various methods. Doses varying between 40 to 180 mgm. injected intravenously slightly slowed the heart rate and decreased the force of contraction. But small doses (2 to 5 mgm.) produced only a slight slowing, present even after atropine and section of the vagi. An intravenous dose of 180 mgm. arrested heart of a dog in systole within about five minutes. The output of the heart decreased after small doses (2 to 10 mgm.) within a few minutes. Excised rabbit's heart showed a gradual slowing with a deficient diastole and the heart did not regain its original condition on the removal of the drug.

Oral administration of the glucoalkaloid as well as an infusion of the drug to dogs was followed by vomiting and diarrhoea. The stools contained mucus and traces of blood.

Prolonged administration of the glucoalkaloid to white rats resulted in a slight increase in their daily output of urine.

The alkaloidal substance from papaya seeds.

Investigation into the chemistry and pharmacological action of the new alkaloidal substance, m.p. 165°C, isolated from *Carica Papaya seeds* is continued. It has been found that it reacts with benzoyl chloride to form a benzoate, m.p. 125°C., whose analysis agrees with that of the monobenzoyl derivative. On bromination it gives a bromo-derivative m.p. 183-86°C. When oxidised with neutral potassium permanganate, it yields benzoic acid. Hydrolysis with alkali splits it up into components, of which one melting at 149°C has been obtained. This component undergoes acetylation to a product with m.p. 131°C. The alkaloidal substance, m.p. 165°C, reacts with methyl iodide to form a substance, m.p. 103-5°C, which appears to be a quaternary base. All these substances are under investigation. An alkaloidal substance, m.p. 165°C, with the properties described does not seem to have been recorded in the literature. Hence the name "Carpasemine" has been provisionally suggested for it. Further work on the constitution of the product is in progress. It is hoped that the study of Carpasemine would be interesting from the point of view of the relationship of structure to pharmacological action. It may also throw some light on the constitution of "carpain", the alkaloid isolated from the leaves (but whose structure is not fully known) in case both these alkaloids are structurally related.

Toxicity tests of carpasemine were carried out on frogs. But doses upto 40 mgm. of the substance given orally and hypodermically produced no noticeable effects in frogs weighing from 50 to 350 gms.

Ringer's solution containing the substance (1 in 5000) when perfused through frogs hearts in situ and excised hearts produced a slight slowing of the rats and diminished excursions. On perfusion of the vessels of the lower limbs of frogs with Ringer's solution containing the substance, the outflow was found to be slightly diminished.

Injection of the "quaternary base" mentioned above, derived from carpasemine produced a curare-like paralysis in frogs.

Other drugs under investigation.

Preliminary study of *Daemia Extensa leaves* and *Erythrina Indica seeds* was continued. An extract obtained from the seeds of Erythrina Indica when injected into frogs produced curare-like action. Attempts are being made to isolate the active substance. The essential oil from *Sphaeranthus Indicus* as well as the amorphous "alkaloid" from *Vinca Rosea, Linn.*, is being studied,

MATERNAL MORTALITY.

32. Enquiry into anaemia of women in Assam under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta.

During the year the work on the enquiry was carried out mainly along three lines, *viz.* (1) animal experiments, (2) determination of normal haematological standards and (3) investigation of anaemia cases.

Animal experiments.

This year animal experiments in relation to the problem of anaemia were conducted mainly along two different lines.

In the first type of experiments, the object was to study the effect of sulphapyridine on the haemopoetic system; for which purpose 10 monkeys living on the ordinary stock diet of the animal room were used. The drug was administered, suspended in water, through a stomach tube to these animals on empty stomach in varying doses. One of these monkeys, however, died during the course of experiment before any clear-cut results were obtained; no obvious cause for death was discernible, even on post-mortem examination. Of the remaining 9 animals, 4 monkeys received a daily dose 0.06 gm. per Kg; 2, a daily dose of 0.12 gm/Kg; 2, a daily dose 0.17 gm/Kg, and 1, a daily dose .08 gm/Kg for periods varying in individual cases from one week to two weeks. In all these cases, a distinct lowering in the level of Hb. and R. B. C. and the examination of bone-marrow from femoral puncture supplied evidence of a toxic depression of haemopoetic function. These experiments also suggested that the intensity of the toxic depression depended not only on the dosage of the drug but also in some way on the individual susceptibility of the animals as there were considerable variations in the results. These experiments are being repeated with the monkeys on an experimental diet.

The other line of work was designed to produce anaemia artificially in monkeys by injection of serum containing a specific haemolysin. This was done by injecting washed red corpuscles of a monkey intravenously into a rabbit; after a few of these injections, when the rabbit's serum was found to contain the specific anti-monkey haemolysin, the rabbit's serum was injected into a monkey intravenously in varying doses. The result obtained so far shows that it is possible to produce a condition of micro-spherocytosis, attended with increased fragility and positive indirect van den Bergh as well as a condition of pseudo-macrocytosis during the period of regeneration from anaemia.

More recently experiments have been undertaken in monkeys to produce a state of chronic anaemia, resulting from chronic malarial infection, artificially induced in these animals.

Determination of normal haematological standards.

Last year while investigating the problem of anaemia in a series of 192 Khasi women in Shillong it was soon realised that some idea of normal haematological background of the population sampled was necessary and, with this end in view, 27 normal Gurkha soldiers were examined towards the close of the investigation. This year, the question of normal haematological standard among hill-tribe was gone into in more detail and complete blood examinations were made in a series of 69 adult males, living and working in and about Shillong. This sample was a heterogeneous mixture of 3 main groups, *e.g.*

coolies, agricultural labourers, and Gurkha soldiers. Although the data obtained have not yet been fully analysed, it is obvious from the findings of this year, as well as of the previous year that the mean corpuscular haemoglobin in each of the groups is definitely higher than that of Calcutta 'normals'; but what is not so obvious is whether the mean corpuscular volume is equally raised in comparison with the Calcutta 'normals'. For, if attention is confined to Gurkha soldiers, it is found that last year the mean haematocrit level was 51.58, whereas this year this was 45.3. Last year, was used a centrifuge belonging to the Shillong Pasteur Institute, whereas this year, a centrifuge which was taken from the School laboratory, after it had been previously checked against the standard machine used in the Anaemia Department of the School. If the difference in the haematocrit readings and consequently in the mean corpuscular volumes of two years is found significant, the question will naturally arise whether such difference could be set down to the difference in the packing capacities of the two centrifuges or to the difference in the nature of the populations sampled or to some as yet unknown factor, operating differentially during two periods of experimentation. This naturally will form a fit subject for further investigation.

INVESTIGATION OF ANAEMIA CASES.

Under the Anaemia Department of the School, there are always some 10 to 12 cases of tropical macrocytic anaemia in hospital, available for investigation throughout the year. Preparations of liver, commercially made, especially those in India, as also those evolved by the laboratory process in the School are frequently tried by way of experiment on these patients. In checking and evaluating the results of this experimental treatment and in assisting in various haematological technique like blood grouping and blood transfusions, much of the work of the enquiry becomes intimately interwoven into the general texture of research work on anaemia in the School.

Since January, 77 complete blood counts on monkeys, 69 on 'normals' in Shillong and 362 on anaemic patients have been done under the auspices of the enquiry.

33. Enquiry into the anaemia of pregnancy in Kanara District under the Director of Public Health, Bombay.

The work of the enquiry was continued on the same lines as indicated in the report of the previous year. During the year 308 bloods distributed as shown in the following statement were examined.

(1)	Pregnant or just delivered women new cases	93
(2)	Repeated examination of the old pregnant cases ..	71
(3)	Non-pregnant anaemic looking women new cases ..	25
(4)	Repeated examinations of non-pregnant old cases ..	15
(5)	Anaemic men new cases	29
(6)	Repeated examinations of anaemia men old cases ..	20
(7)	Normal cases, men, women and children from villages ..	55
		308

Results of blood examinations—

Pregnant women 93 cases.

Classification according to total R. B. Cs count,

	No. of cases
Below 0.5 million per 1 c.mm.	1
Between 0.5 —1 million per 1 c.mm.	11
,, 1 to 1.5 ,, ,, ,, ...	10
,, 1.5 to 2 ,, per 1 c.mm.	9
,, 2 to 2.5 ,, ,, ...	12
,, 2.5 to 3 ,, ,, ,, ...	11
,, 3 to 3.5 ,, ,, ,, ...	16
,, 3.5 to 4 ,, ,, ,, ...	14
,, 4 to 4.5 ,, ,, ,, ...	8
,, 4.5 to 5 ,, ,, ,, ...	1
Above 5	0
	93

Classification according to Hgb content.

Below 5 grms. per 100 c.c.	18
Between 5 and 7 grms. per 100 c.c.	13
,, 7 and 10 grms. per 100 c.c.	18
,, 10 and 13 grms. per 100 c.c.	31
Above 13 ,, ,, ,, ...	13
	93

These 93 pregnant women whose bloods have been examined came mostly from the town of Sirsi itself and neighbouring villages. Almost all the cases of pregnant women that came to the dispensary were taken up for examination without any selection for anaemia. The statements above will therefore show that nearly half the number of cases have total red cell count of above 3 million and haemoglobin content of more than 10 grms. per 100 c.c.

Amongst these 93 cases there were 26 cases of macrocytic type of anaemia and 5 cases of microcytic type.

In the macrocytic type the least total R. B. Cs count was 0,475,000 and the highest 2,790,000. Least haemoglobin was 2.66 grms. per 100 c.c. and highest 12.84. Least cell volume was 5.76 and highest 31.82 parts per 100 c.c.

A good number of these cases came in, in an advanced stage of the disease. There were six fatalities in these 26 cases. Two of them died on the day of admission and one on the 4th day. One was in the hospital for more than a month. She did not respond to injections of liver and died at home 2½ months later. The remaining two cases left the hospital soon after admission and on enquiry are reported to have died 1½ and 3 months later.

All the cases of this type were treated with injections of various brands of liver extracts. Some of these got marmite and a few dried separated milk.

Apart from these 26 cases there were six others which though not definitely macrocytic were pointing to it. Two of these ultimately did go to that stage and died.

Attempt was made to mark out early cases of these types and put them on either marmite or dried milk alone, to find out if possible, if such dietetic treatment would help to prevent the advance of this disease. But the cases so obtained were too few to come to any definite opinion on that point.

Malaria and worm infection is very common in this district. Large number of population have enlarged spleens and the worm infections. Amongst these 26 cases, though none showed parasites of malaria in their bloods, 14 of

proportional. Unlike Willmer and Kendal the mitotic cell count in the present series has been fewer in number. Otherwise the main findings in this series are in conformity with those observed by Willmer and Kendal. Experiments for the biochemical estimation of the culture media before and after cultivation have been commenced. The amount of nitrogen involved in the process is very small and if the experiments succeed further evidence as regards utilisation will be available.

Several cultures were put up by the flask method, a few Carrel flasks being available recently. For this Carrel flasks of the type D.3.5 were used. Explants were taken from the chick embryo heart and was cultivated in a medium consisting of a solid and a liquid phase. Fowl plasma constituted the solid and embryo extract diluted with Tyrode solution the liquid phase. The liquid phase is renewed when necessary without disturbing the growing cells, which enables the cultures to go on growing for much longer periods than is possible in the hanging drop method and the growing cells can also be studied longer.

37. Enquiry into medical mycology under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta.

Twenty cases of moniliasis of the skin and mucous membranes have been studied to identify the species commonly found here. Some of the cases affecting the skin simulated ringworm. The results are being analysed for publication.

Five cases of Tinea imbricata were studied as regards the species of the causative organism responsible for the condition in India. The material was collected from five subjects in different villages in Assam. A detailed morphological and cultural study was done. Morphologically the five fungi were identical. But the cultural characters showed some differences both in the nature of the growth and colour. These differences, however, can be eliminated with repeated sub-cultures in glucose agar medium under constant oxygen tension. This observation is in agreement with the previous findings and confirms the view that the fungus is probably an *Achorion* and that one species *Achorion concentricum* is the sole cause of tinea imbricata in the East.

The work has been completed and the paper published on an improved technique for obtaining pure cultures of *Epidermophyton floccosum*. By the addition of a very small quanitty of gentian violet to Sabourand's proof medium most of the contaminations can be killed and pure cultures more easily obtained.

A number of cases of verrucose tuberculosis of the skin were treated successfully by the local application of a paste containing salicylic acid and creosote with glycerine as a base. The paste is applied with a piece of lint and the part is kept bandaged. In 48 hours time the whole of the diseased tissue falls off leaving a healthy granulating surface underneath. The paper on this was published in the beginning of the year.

Sixty cases of cheiropompholyx have been studied in detail to discover the probable aetiological factors responsible for this condition. The disease is common in the tropics and affects all races and classes and causes a good deal of suffering and loss of time. Although cheiropompholyx is said to be caused by ringworm infection, the present investigation shows that at least half the cases had nothing to do with ringworm. The probable aetiological factors in these cases are focal sepsis in the throat, teeth, nasal sinuses or in the bowels. The results are being analysed for publication.

Ringworm infection of the scalp is principally a disease of children and frequently clears up spontaneously with the advent of puberty. This spontaneous cure has been attributed to gonadal activity at that period. Whooping cough in children has been claimed to be benefited by the injection of gonadotropic hormones. This aetiological analogy led to the clinical trial of gonadotropic hormones in ringworm of the scalp in children, in 20 cases. The results may be briefly stated as follows :—(*a*) This treatment is useful in Microsporosis and in the infection by *Trichophyton crateriforme*. (*b*) In *trichophyton violacium* it proved to be useless.

Since the publication of the work on fungicidal and fungistiatic properties of certain dyes and chemicals ; a combination of three dyes, acriflavin, gentian violet and brilliant green has been found to be very effective in the treatment of burns, in the United Kingdom. This combination is being tried clinically in certain septic conditions of the skin and also on fungi in *vitro*.

A very large number of cases of dermatitis still remain to be properly classified. Seborrhoea is said to be one of the common causes of generalised dermatitis. There are no fixed criteria for diagnosing chronic seborrhoeic dermatitis, especially when it is generalised. An investigation has been undertaken to find whether the serological reaction (complement fixation test) can be of any help. An antigen prepared from the culture of *Malassezia ovâle* has been studied and 90 per cent have given positive reactions. This antigen does not give any reaction to the cases of Pityriasis versicolor caused by an allied species *Malassezia furfur*. This investigation is not complete.

Four cases of nodosities of the axillary hair have been studied and the causative organism, a *Nocardia* has been isolated. As it is likely to be the causal factor in erythrasma of the axilla and pubic regions, this possibility is being investigated.

Study of immunity to the ringworm fungus (*Epidermophyton floccosum*) in animals :—(*a*) Small laboratory animals such as rabbits and guineapigs were found unsuitable for the experiments. (*b*) Young monkeys are susceptible to the ringworm infection. (*c*) Infection takes place if a culture of the fungus is rubbed over a scarified area of the skin. (*d*) Spontaneous cure takes place in about 2 months. (*e*) No further infection can be given after the first infection, up to a period of one year. (*f*) Intraperitoneal injections of the serum from the immune monkey give protection to other monkeys. (*g*) Investigations are now being made to find out whether infection of hair ringworm such as *Trichophyton violaceum* gives cross immunity against *E. floccosum* and if that is possible the investigation may be extended to bigger animals, for example horses to try to produce an immune serum to be tried on human beings.

Leucoderma—(*a*) Treatment of leucoderma was carried on throughout the year by the injection of sterile bouchi oil. As the pure oil is very painful an improved technique was adopted by the addition of 4 per cent double-distilled creosote to the sterile oil. This has been found a distinct improvement and quite a large number of cases has been successfully treated in the year. (*b*) The histopathological study of the regenerating patches has been undertaken to find out the cells responsible for producing the melanin.

38. Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta.

Observations on the spread of filarial infection in an endemic area.

The tea gardens of Lakhipur and Binakandy in Cachar district in lower Assam comprise nine villages. These are endemic for filariasis, the infection being mainly *Wuchereria malayi*. It is said that these villages were quite free from filarial infection till about 30 years ago when the infection was introduced by imported labour from Behar. Buckley observed the infection for the first time in 1934 in these villages, but Fraser made a thorough study of the infection in 1936 and found a gradual increase in the infection in subsequent years. It was with a view to observing the rate of spread of the infection and also to collect adult specimens of the parasites that a survey was undertaken during the early part of this year.

The types and number of filarial diseases observed were as follows :—

Elephantiasis legs 89, Elephantiasis arms 16 and Elephantiasis legs and arms 4. Lymphangitis, abscess and adenitis were common. There was no case of genital affection nor of chyluria in the population. One case of hydrocele was imported from Muzafferpur (Behar).

Blood smears taken at night of 2,445 persons from these villages were examined for microfilariae ; of these, 115 showed microfilariae in the peripheral blood, showing a gross infection rate of 4.7 per cent. Of these 115 carriers, 5 were *Microfilaria bancrofti* infection while the rest were *Microfilaria malayi* infection. The former infection was found only in one village, *viz.*, Lakhipur. In Lakhipur, out of six positives five were bancrofti infection and one was malayi infection.

The disease rates for the villages compared with the figures reported by Buckley for 1934 and by Fraser for 1936 and 1938, respectively, show that the infection remains the same while disease shows an increase in 1941. The increase in the disease appears to be gradual. The infection does not so far appear to have extended to neighbouring villages.

A mosquito-survey carried out in these villages showed 15 species of mansonoides, anophelines and culicines.

One remarkable fact observed during the survey was that villages situated in the midst of extensively cultivated land, were practically free from filarial infection ; on the other hand, villages situated in the midst of swampy areas, showed a high incidence of filarial infection.

Filarial survey of Santal Parganas and its environs.

A cyst in the eye with filarial worms had been reported previously in Rampore-Haut, which lies on the eastern border of Santal Parganas. No other case of filarial infection has been reported so far from this area. With a view to ascertaining the nature of infection in this area, a survey was undertaken and blood specimens were collected from the indigenous population in the area. The district is an undulating and elevated region between altitudes of 500 to 1,000 ft. The average annual rainfall is about 60 inches. The population varied from 10,000 to 30,000 in Giridih, a mining centre. The percentage of infection ranged from 3 to 20 per cent. In all these places the infection was found to be that of *Mf. bancrofti*.

Survey of Thana district in Bombay Presidency.

A filarial survey of Thana district in the Bombay Presidency was also undertaken with a view to ascertaining the species of parasite prevalent there. The infection was *W. bancrofti.*

Duration of life of Mf. bancrofti in hydrocele fluid.

The duration of life of *Mf. bancrofti* in hydrocele fluid was determined by transfusion experiments, and it was observed that the average life was about 18 days. Work on these lines is being continued.

Treatment of hydrocele.

A number of drugs, such as Quinine Urethane, Sod. Morrhaute, Sod. Salicylate, Morestien's fluid and Lactic acid, were investigated for the treatment of hydrocele by injecting the drugs into the hydrocele cavities. Quinine Urethane has so far given the best results.

Treatment of filarial infection.

Further investigations in the treatment of filariasis were carried out with the following substances :—

1. Filarial allergen, 2. Proteose, 3. Ichthyol, 4. *Mimosa pudica* extract and 5. Fantorin (an organic trivalent antimony compound manufactured by Glaxo Laboratories).

With the exception of the allergen and ichthyol none of these substances showed any appreciable effect on the infection. Allergen appears to be helpful in cases where allergic symptoms are prominent. Oral administration of ichthyol gave satisfactory results in a few cases of chyluria.

39. Enquiry into filariasis under Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Vizagapatam.

The work done during the year was as follows :—

Experimental filariasis.

The study of the development and migration of the filarial worm in its primary host was carried out in the lizard *Calotes versicolar* which is found to be naturally infected with the filarid *Conispiculum guindiensis.* Eight developmental stages were studied, before the developing filarid gave rise to embryos in peripheral blood in about 70 to 90 days. At this stage the worm was still half of its full grown size. There did not seem to be any tissue reaction on the part of the host around a live worm.

Bacteriology of filarial conditions.

A study of the bacteriology of the tissue fluids obtained from 25 proved filarial cases showed that the inflammation was sterile in the majority of cases, in a few cases organisms of low virulence such as Staphylococcus albus and citreus being isolated.

In 63 cases of live-births 36 full term and 27 prematures) and four dead births (3 full term and 1 premature) there was evidence to throw doubt upon the maturity of the infant as recorded.

In regard to sex. the distribution among live-births was as follows: 1,242 males and 1,126 females (for full term infants the proportion was 1,146 males: 1,035 females, and for premature births 96 males: 91 females). In the case of still-births the proportion was 23 males: 30 females (full term births 12 males: 20 females, and premature births 11 males: 10 females). In 201 cases the sex of the infant was not stated (137 full term live births, 18 full term still born, 32 premature live, 14 premature dead).

Among the 2,537 live-births, 281 deaths were recorded during the first year of life, 191 of full term infants and 90 of prematures, using maturity as recorded. Excluding the 63 cases of doubtful maturity, the total number of deaths became 266, 185 full term infants and 81 of prematures. In the table below are shown the figures relating to both agreed and recorded maturity and to the record or survival of death or the infant, whether full term or premature.

	Agreed maturity		Recorded maturity	
	Full term	Premature	Full term	Premature
Died within a week ..	42	45	43	51
No information within a week ..	171	14	171	14
Died within a month ..	39	18	42	20
No information after a week ..	185	21	187	22
Died within 3 months ..	29	14	31	15
No information after a month ..	371	19	377	22
Died within 6 months ..	33	2	33	2
No information after 3 months ..	231	10	234	11
Died within 9 months ..	22	2	22	2
No information after 6 months ..	244	10	250	11
Died within a year ..	20	..	20	..
Alive upto a year ..	540	24	549	35
No information after 9 months ..	355	13	359	14
Total ..	2,282	192	2,318	219

From the above table it is calculated that for infants of agreed maturity the death rate within a week of birth lies between 19.9 and 18.4 *per mille* of live-births for full term infants, and between 252.8 and 234.3 *per mille* of live-births for premature infants. The combined rate lies between 38.0 and 35.2 *per mille*. The still-birth rate is 20.6 for full term infants *per mille* of live-births; and for premature infants 177.1 *per mille*. The combined still-birth rate is 32.7 *per mile*.

It will also be seen from the preceding table that records of live-births were incomplete to an extraordinary degree. In only 584 cases was the infant recorded as live at the end of one year. In 185 cases contact was lost within a week of birth; with a further 209 cases within the first month of life. Records of a further 399 infants terminated between the second and third month; 245 were lost sight of in the second trimester of life, and 261 in the third trimester. A further 373 infants were lost contact with during the fourth trimester.

The recorded causes of death are shown in the table given below :

	By recorded maturity.	
	Full term	Premature
Smallpox	12	..
Chickenpox	1	..
Measles	5	1
Whooping cough	2	..
Diphtheria	3	...
Tetanus	10	1
Dysentery	2	..
Cholera	2	..
Birth injury	..	1
Pneumonia	27	6
Convulsions	10	1
Meningitis	1	..
Congenital debility	11	3
Neglect	6	2
Tuberculosis	5	..
Congenital syphilis	2	..
Typhoid	1	..
Diarrhoea enteritis	17	5
Cirrhosis of the liver	1	..
Congenital malformation ..	2	1
Accident	1	..
Prematurity	..	30
Asphyxia	3	..
Intoxication due to maternal toxaemia	..	5
Haemorrhage of the umbhicus ..	1	..
Starvation	1	..
Marasmus	1	1
Respiratory disease	1	1
Sepsis	2	..
Not known	65	32
Total ..	195*	90

*In 4 cases, 2 causes of death were recorded.

To obtain causes of death of infants of agreed maturity, the following deductions should be made :

Full term		Premature	
Convulsions	.. 1	Unknown	.. 4
Congenital debility	.. 2	Intoxication due to maternal toxaemia	.. 1
Neglect	.. 1	Congenital debility	.. 1
Not known	.. 2	Congenital malformation	.. 1
		Prematurity	.. 2

The result of pregnancy for all single births of agreed maturity for the different years are as follows :—

Table

Result of pregnancy	1938	1939	1940	Total
F. T. alive	710	816	746	2,282
F. T. dead	14	13	20	47
Prem. alive ..	57	65	70	192
Prem. dead ..	6	7	21	34
Monster ..	nil	nil	1	1
Macerated foetus ..	9	3	6	18
Abortion	19	27	35	71
	825	931	889	2,645

In 1940 there was a slight rise in the premature still birth rate: apart from this no variation was detectable.

Considering these results of pregnancy by centres the numbers are:

Centres.

Result of pregnancy	1	2	3	4	5	6	7	Total
Full term alive ..	226	35	652	353	160	306	550	2,282
Full term dead ..	4	..	5	14	4	5	15	47
Premature alive ..	6	6	62	14	29	42	33	192
Premature dead ..	1	1	2	5	8	11	6	34
Monster ..	..	..	..	..	..	1	..	1
Macerated foetus ..	..	..	3	3	2	2	8	18
Abortion ..	9	1	33	6	13	3	6	71
Total ..	246	43	757	395	216	370	618	2,645

Premature births were more numerous in centres 5 and 6 (Clive Jute Mills and Kidderpore Centres).

Abortions were more numerous in the records of centres 5 and 3 (Clive Jute Mills and the Institute Centre).

Although detailed analysis has not been made, it is apparent that because of deficiencies in the records utilised, the number of factors whose effect on the result of pregnancy and survival of the infant can be assessed, will be limited.

For instance record of diet was poor, so much so that the adequacy of the diet especially in regard to protein and vitamin sufficiency could be estimated in only the number of cases tabulated below :—

	Capable of estimation.	Not capable of estimation.
Protein ..	259	2,386
Vitamin A ..	94	2,551
Vitamin B ..	147	2,498

The tabulation and statistical interpretation of the data for this study is being conducted by Dr. C. Chandrasekar.

OTHER RESEARCHES.

36. Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

The enquiry has mainly been concerned with the study of the utilisation of proteins, an essential food factor, by chicken heart fibroblasts grown in cultures, according to the hanging drop technique.

Pure proteins as well as amino-acids are known to be unsuitable and result in poor growths. The remarkable growth promoting property of embryo extract is well known and Baker and Carrel have domonstrated that the protein factor of the extract is responsible for this. In their subsequent work they succeeded in obtaining very good results by using the higher fractions of protein digestion, *e.g.*, the proteoses. Willmer and Kendal however pointed out that in the absence of plasma in the medium, proteoses were not utilised to any great extent and resulted in poor growths. The latter isolated another protein fraction which is a heteroproteose and was named substance X by the authors, and claimed it to be a powerful stimulant to multiplication and growth of chicken heart fibroblasts, even in the absence of plasma in the medium.

Samples of proteoses and substance X have been prepared in our laboratories from Witte's peptone and blood fibrin, according to the technique described by Willmer and Kendal. These were dissolved in specially prepared Tyrode solution so that the final solutions contained the protein fractions and the various ingredients of the Tyrode solution in the usual strength. The nitrogen content of the solutions and their pH were determined. For the cultures explants were taken from the heart of chick embryos of 7-10 days incubation, the media consisted of the solutions of the protein fraction having different nitrogen concentrations with or without the addition of plasma. Control cultures were also put up where the solution of the protein fraction was omitted from the media.

The proteoses in the absence of plasma presented poor growths whereas on the addition of plasma there was a considerable improvement in the picture. This tends to point out that the proteoses are further split up by plasma enzymes into more suitable fractions which are better utilised by the fibroblasts.

Cultures having substance X dissolved in Tyrode solution commenced to grow early, the cells multiplying at a rapid rate, and a wide area is covered by the end of 30-36 hours, subsequently there is a fall in the rate of growth and further growth appears to be negligible after the end of 40-48 hours. If kept longer the cells tend to acquire a rounded appearance. The cultures were fixed and stained after 40-48 hours incubation. The growth areas were charted on graph paper by camera lucida drawings. Three methods were practised for measuring the total areas of growths, these were (1) direct counting (2) weighing and (3) planimeter recording. The most suitable of these three methods will ultimately be adopted. Some microphotographs were also taken from interesting specimens. Substance X was found definitely to be a powerful stimulant for chicken heart fibroblasts and compared favourably with embryo extract, but unlike embryo extract with substance X the cells appeared scattered and rather undernourished. The addition of plasma in the media improved the appearance to a great extent and the cells were better nourished and grew in a compact mass. It appears therefore that the action of substance X is more of the nature of a growth stimulant than an adequate source of nitrogen supply. Samples of substance X solutions having higher nitrogen concentration generally showed more abundant growth but this increase was not strictly

Action of drugs.

The effect of about a hundred drugs 'in vitro' on the infective larvae was studied. The results were generally disappointing since the drugs acted only in concentrations that are impossible 'in vivo'. To approximate 'in vivo' conditions as far as possible, in the latter experiments serum of animals and filarial patients after previous medication with the drug in question was tested. Further work in this line is in progress.

Experimental transmission.

The susceptibility of the common laboratory animals to the bancroftian filariasis was investigated by various methods. The results appeared to show that the rabbit, guineapig, white mice and dogs were all non-susceptible.

Diagnostic methods.

An antigen was prepared out of *Conispiculum guindiensis* and an attempt was made to assess its value as a substitute for Dirofilarial antigen in the diagnosis of human filarial conditions by the intradermal method. The results were indefinite. Further work is necessary for a definite statement. Attempt to prepare an antigen from Bancroftian microfilaria was not successful.

Treatment of cases.

The results of treatment of filarial conditions with a number of drugs showed that none was useful in destroying either the adult parent worm or the microfilaria in peripheral blood. Local infiltration treatment along the line of diseased lymphatics with a solution of salts of Hydnocarpus oil was of some value. Serum treatment, immunisation with filarial fluids etc., were all of no value.

40. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras.

Vaccinia virus.

Experiments initiated last year to ascertain the relative value of chick membrane lymph and routine calf lymph in the prevention of smallpox were continued during the year. Twentysix villages situated within a range of 15 miles from the Institute were selected for this study. In these villages, no intensive vaccination campaign had been undertaken in the last 3 years, and therefore, sufficient unprotected population was available.

In each village, alternate cases were vaccinated with chick membrane lymph and calf lymph. The results of one full year's observations were forwarded to the Public Health Commissioner with the Government of India, in June 1941. It was found that in 748 cases of primary vaccination with membrane lymph, a case success rate of 89.2 per cent and insertion success rate of 73.2 per cent were obtained. The success rates for calf lymph were 99.1 per cent and 97.6 per cent respectively in 744 cases vaccinated. While the rates obtained for membrane lymph are low as compared with calf lymph, they were more or less the same as recorded in England, viz. 85 per cent with membrane lymph against 95 per cent with calf lymph (Ref. letter dated 6th

July 1939 from the Deputy Chief Medical Officer, Ministry of Health, to Major-General Sir John Megaw). The general appearance of vesicles was the same as observed with calf lymph as regards their development, inflammatory reactions, etc. No untoward complications have been noticed so far.

Revaccination.

In 1,078 cases of re-vaccination done with membrane lymph, a case success rate of 11.8 per cent was obtained. It is to be noted that these cases were vaccinated previously with calf lymph. However, in 1,050 cases re-vaccinated with calf lymph, a case success rate of 54.8 per cent was obtained. The difference in the two rates is, therefore, well marked. The high success rate of 54.8 per cent in re-vaccination with calf lymph is of particular interest, in view of the low percentage of case success rates in the Province as a whole, viz. 22 per cent.

During the year under report, none of the villages were infected from smallpox. These field observations are being continued.

The keeping qualities of chick membrane lymph were investigated in detail. It was found that stored at —5°C, the potency was unimpaired at the end of one month's storage in almost all the cases. A few batches of lymph were, however, found to have retained their potency for 105 days. As was noted in previous years, there appeared a periodical fall in potency as the serial passage on egg membrane progressed. In order to ensure uniformity of lymph for field use, it was considered advisable to issue pooled lymph from at least 3 serial passages. It may be of interest to note that in the majority of cases when unused lymph was returned from the field, it was found potent by the usual laboratory tests.

So far in the lymph issued, no glycerination was adopted. At present, the keeping qualities of chick membrane lymph in 50% glycerine-tyrode are being investigated. So far, no difference in the potency in the two sets has been noticed.

Rinderpest virus.

Attempts were made to cultivate rinderpest virus on the chorio allantoic membrane of developing chick. Both goat and bull virus strains were studied. No definite adaptation to chick membrane has been observed so far. The attempts will be continued.

Influenza virus enquiry.

A supplementary grant was made during the year for preliminary studies on the virus of influenza. A detailed programme of work was submitted to the Scientific Advisory Board which included importation of a strain of virus from abroad for experimental comparative tests. It was not considered feasible to proceed with this aspect of the question at the present moment and attempts are being made at present to isolate virus of influenza from clinically diagnosed cases both during epidemic and non-epidemic periods.

Five cases of sporadic influenza were investigated and attempts were made to isolate the virus by Burnet's technique of direct inoculation of filtered throat washings into the amniotic sac of the developing chick embryo and also by serial passages in mice directly with throat washings as well as with a passage material obtained from inoculated egg. 5 to 10 serial passages were made in

each case, but no evidence of infection either of chick embryo or of mice was obtained.

Throat washings obtained from 6 cases during a sharp outbreak of mild and uncomplicated influenza in an institution were passed serially in developing chick embryo. There is so far no evidence of infection of the chick. Attempts were also made to cultivate the virus in tissue-cultures according to the technique of Rivers adapted by Hyde. The work is in progress.

41. Protozoal parasites enquiry.

(This enquiry was under the direction of Lieut.-Colonel H. W. Mulligan, I.M.S., Director, Pasteur Institute, Coonoor, in January and February. On Lieut.-Colonel Mulligan's departure on active service, it was placed under the administrative control of the Director, Nutrition Research Laboratories, Coonoor, and the technical direction of Dr. Paul F. Russell, International Health Division, Rockefeller Foundation. In September the enquiry was transferred to Shillong, under the direction of Lieut.-Colonel L.A.P. Anderson, I.M.S. (R), Director, Pasteur Institute, Shillong.)

The work of the enquiry during last year has been directed mainly to trying to find the transmitter or transmitters of the plasmodium of the monkey (*P. knowlesi*). In addition to this some work on the immunity in monkey malaria has also been done. Although the results obtained with the species of mosquitoes experimented with have all been negative, some new observations have been made especially with regard to the conditions under which the exflagellation of the parasite takes place. These will be of value for future work if and when it is resumed.

EXPERIMENTS WITH THE TRANSMISSION OF PLASMODIUM KNOWLESI.

In the absence of any clue as to development, various tree-hole breeding species of mosquitoes were experimented with, as the parasite in nature in Malaya is found in jungle-inhabiting monkeys. In addition some other mosquitoes made available through the courtesy of Dr. P. F. Russell of the Rockefeller Foundation were made use of. The results obtained are summarised in the table below:

Mosquito species.	Number fed.	Number dissected.	Interval in days between feeding and dissection.	Result.
A. stephensi ..	238	183	13–19	negative.
A. subpictus ..	3	1	15	,,
C. mimeticus ..	54	42	14–19	,,
L. raptor ..	28	22	14–19	,,
A. albopictus ..	212	198	13–20	,,
A. albolateralis ..	22	12	17	,,
A. chrysolineatus ..	48	27	13–18	,,
A. decanus ..	27	14	11–18	,,
A. vittatus ..	4	2	19	,,
A. obturbans ..	49	32	14–18	,,
A. kuchingensis ..	24	22	15	,,
L. flavus ..	76	44	15–18	,,
L. magnus ..	113	59	15–18	,,

There is no available record as to when exflagellation occurs in the case of *P. knowlesi*. The parasite, being a very virulent one to Indian species of *Macacus*, kills off the host in a short time, without allowing sufficient time for the maturation of gametocytes in the host. A large series of trials under various conditions was therefore made. In some instances trials were made with blood every day from the beginning of infection to the death of the animal or till the parasite disappeared from the blood. The following table summarises the results obtained.

Temperature °F	Humidity %	Time of exposure of the smear.	Result.
90	60	45 mts.	Negative
90	66	30 mts.—24 hrs.	,,
90	72	30 mts.— 3 hrs.	,,
90	79	1— 3 hrs.	,,
92	61	1—24 hrs.	,,
92	66	1— 3 hrs.	,,
92	73	2—30 mts.	,,
92	76	7—60 ,,	,,
92	79	5—30 ,,	,,
92	82	2—60 ,,	,,
92	86	5—60 ,,	,,
93	70	3—45 ,,	,,
93	75	3—30 ,,	,,
93	76	3—30 ,,	,,
94	73	5—60 ,,	,,
81	92	5—60 ,,	,,
81	96	2—60 ,,	,,
82	88	2—60 ,,	,,
82	92	5—60 ,,	,,
82	96	5—60 ,,	,,
84	77	5—60 ,,	,,
84	85	5—60 ,,	,,
84	88	5—60 ,,	,,
84	92	5—60 ,,	+
86	82	5—60 ,,	Negative.
86	85	5—60 ,,	+
86	88	20—60 ,,	+
86	92	5—60 ,,	+

This is the first successful record of exflagellation, as far as published results go, with undoubted *P. knowlesi*, as the previous records have concerned mixed infections. The necessary conditions appear to be a lower temperature and a high degree of humidity. In each of these trials several slides were made at the same time and stained and examined. The first positive results were obtained in the night films and so a comparative study of the films taken both during night and day under identical conditions was made. It was seen that if the temperature and humidity conditions were kept constant, positive results were obtainable in both series.

Immunity studies with Plasmodium knowlesi.

Previous experiments done by this enquiry have shown that the sera of animals suffering from malaria exert a protective action. Whether these operate by sensitising the cells or by killing off the parasites is not known. Certain experiments were carried out to throw light on this question. Known quantities of parasites were incubated with immune serum at 37°C. for two hours and inoculated into animals which had previously been sensitised by inoculation of immune serum from animals suffering from *P. knowlesi* infection, for six days. Only a small number of animals were used, and the results were inconclusive. Out of six animals, one escaped infection. Those that became positive were treated with immune serum. Two monkeys died on the 7th day and the other three in 11-13 days. A larger series of animals would have to be used before conclusions could be drawn.

Miscellaneous.

The existence of *P. inui* as a natural parasite in *sinicus* monkeys at the foot of the Eastern Nilgiris has already been reported. Further examination of monkeys from other parts, viz., the Western Nilgiris (Gudalur), has shown a similar infection to be present there also. Five animals out of a total of 7 examined showed the parasites, which appeared to be morphologically similar to *P. inui* as previously described.

The enquiry was under the administrative control of the Director, Nutrition Research Laboratories, and under the technical control of Dr. Paul F. Russell of the Rockefeller Foundation, for the period covered by this report. Thanks are due to both these officers for giving their valuable time. The staff of the enquiry were transferred to Shillong in September 1941.

42. Kala azar enquiry.

Till July 1941 this enquiry was conducted in Bihar under the direction of Dr. R. O. A. Smith. Then the enquiry was transferred to the School of Tropical Medicine, Calcutta, under Dr. L. E. Napier, and from Calcutta it was again transferred in October to the Pasteur Institute, Shillong, under Lieut. Colonel L. A. P. Anderson I.M.S. (Rtd.), Director of the Institute.

During the earlier part of the year work was limited to a study of the bionomics of *P. argentipes* with reference to breeding sites, range of flight and attraction to various domestic animals.

Breeding sites.

From every likely and unlikely spot in the vicinity of two cattle sheds, samples of earth were collected for examination for larvae. Of 58 samples from the vicinity of one shed, larvae were found in 16 ; in two instances larvae in fairly large numbers were found in samples taken 30 yards or so from the shed. From the area surrounding the second shed 17 samples were examined and of these 10 were positive. Identification of the larvae from these sources revealed that from sites close to dwellings, *P. minutus* (sp) predominated ; *P. argentipes* larvae were found in samples further removed from the houses, and usually under heavy vegetation. There was not much variation in the type of vegetation present to determine whether *P. argentipes* favoured any special

plants; but their presence under bushes over-grown with creepers suggested that they selected places most sheltered from the sun and rain.

Attraction to domestic animals.

By substituting other animals for a cow in its stable, it was confirmed that neither goats nor dogs had any great attraction for this species. It was not possible to determine to what extent the flies infested human dwellings when cattle were removed from the vicinity, owing to the objection of various house-holders to the entry of the collectors to their living rooms. It is interesting to note that *P. argentipes* does not voluntarily feed on dogs; this may be one reason why dogs in India are not found naturally infected with *L. donovani*.

Experiments with marked flies resulted in the capture of such individuals 20 yards or so from the spots where they were released, but, by moving a cow from its usual shed to a clean room some distance away, flies have been found to travel 5 yards or more in search of suitable feeding.

Human transmission experiments.

At the beginning of October 1941 a field station was established at Gauhati, where laboratory accommodation was provided for the enquiry by the courtesy of the Assam Government.

The breeding of sandflies for the work in connection with human transmission experiments were immediately started and 4166 female flies were collected from houses and cattlesheds. From these 4280 flies (2189 females) were bred out. Considering that it takes about five weeks for the flies to complete their life-cycle in ordinary weather and longer in cold weather, this number represents only about half of the progeny from the collected flies.

The collection of flies in nature almost stopped by the end of December as, with the cold, flies had become very rare. The breeding operations were however carried on with laboratory-bred flies by means of an incubator maintained at 28°C, which is the right temperature for the early stages of the flies.

From the bred flies 1184 females were fed on kala-azar cases. The first feed was effected on 29th November 1941 and most of the feeding was done during December 1941. Ten cases were made use of for feeding. The cases were diagnosed by the aldehyde test, eight of them being confirmed by the demonstration of Leishman-Donovan bodies in the peripheral blood.

The technique adopted for keeping the flies alive after the initial feed on kala-azar patients and before they were fed on the volunteers was that advocated by Smith (Indian Journal of Medical Research, October 1940). As he had demonstrated positive transmission in hamsters by flies which were kept alive for 10 days on raisins after the infecting feed, the flies were fed on the volunteers only after a minimum of 9 to 10 days had elapsed after their initial feed on the patient. All the six volunteers received several infecting feeds by flies so kept. Two hundred and thirtysix flies were fed on them, out of which only 17 were actually found infected by flagellates on subsequent dissection (some of them very heavily).

43. Enquiry on the algae present in water reservoirs and their effect on purification methods and on public health under the Director, King Institute, Guindy, Madras.

The work during the year comprised (1) the ecological study of the algal flora of (*a*) the Red Hills Lake in Madras; (*b*) four water supply reservoirs and four mountain streams in Ootacamund; (*c*) the Stanley Reservoir at Mettur; (*d*) the Panamaruthupatty tank at Salem; (*e*) the Rajah tank at Cocanada; (*f*) the Adyar river water at Guindy before and after storage; (*g*) the deep wells supplying water to Dindigul Municipality; (2) the investigation of algicidal measures to combat algal troubles in waterworks; (3) a study of the effect of algal growths on water purification processes; (4) a study of the nature and composition of the bottom mud of reservoirs and their influence on algal productivity; (5) the relation between the algal plankton and the organic matter content in waters; (6) studies on the regeneration of nitrogen compound in the course of the decomposition of dead plankton; and (7) a study of the methods used in the rapid quantitative estimation of algal plankton.

Ecological studies.

Observations continued during the year at Madras and Salem confirmed the previous finding that the organic matter content of water influenced the composition of the plankton algae to a considerable extent. Diatoms were found to occur in large numbers in the Red Hills Lake and in the Ooty reservoirs immediately after a heavy rainfall and a simultaneous increase in the silica content also occurred. As the diatoms increased markedly in number, there was a progressive decrease in the silica content of the water.

A high organic matter content of the water and a high temperature favoured the prolific growth and reproduction of the blue greens *e.g. Microcystis, Phormidium* and *Anabaenopsis*. These forms were found to grow and multiply profusely *even in the total absence of nitrates and phosphates in the waters tested*, suggesting that certain blue green algae can perhaps utilize nitrogen from the atmosphere for their growth and multiplication.

The further study of the Ootacamund reservoirs confirmed the findings already reported. A comparative study was made of these drinking water supply reservoirs at Ootacamund and of the Ooty lake which is not a drinking water source and which being situated within the town, is contaminated with the drainage waters from the surrounding inhabited area and from cultivated lands. Nitrates were present in large quantities in this lake and the algal flora found in this lake showed a corresponding difference in their composition.

Limnological studies.

Limnological studies of the large Stanley reservoir at Mettur were continued. Observations indicate a very clear and well marked seasonal variation in the phytoplankton which are composed largely of *Microcystis, Pediastrum* and *Staurastrum* in early summer, they being replaced by a heavy growth of *Mougeotia* towards the middle of the summer. With the advent, however, of the monsoon and flood conditions, diatoms predominate. No marked thermal stratification was observed. Appreciable amounts of hydrogen sulphide are produced in the bottom layers during summer partly as a result of the decomposition and putrefaction following the death of the algae.

Nearly all the water supply to the Nilgiris is derived from perennial hill streams. These waters were acid in reaction, and contained large quantities of dissolved carbon-dioxide, organic matter and nitrates ; these streams supported a very poor growth of phyto-and zoo-plankton. Nymphs of *May-flies* and *Trichopteran* larvae formed the chief stream fauna.

Algicidal treatment.

Observations on the effect of copper sulphate and chlorine applied periodically to the Rajah tank at Cocanada showed that algal growths could be effectively checked by suitably controlled treatment applied systematically to the incoming raw water. Control measures were further studied in detail at Salem and at Dindigul. At the latter place, the repeated application of small doses of copper sulphate alone (0.2 to 0.4 p.p.m.) was found to be very effective in checking the growth of *Pithophora* which was the predominant type.

The production of sulphuretted hydrogen in the slow sand filters at Salem was considerably reduced by controlled copper sulphate treatment applied to the raw feed water. This also resulted in the increase of the filter runs and in effecting considerable economy in the cost of filtration and chlorination.

Experiments to determine the dose of the proper algicides required for various types of algae were continued. The forms belonging to the group Protococcales (*Ankistrodesmus Pediastrum, Oocystis, Scenedesmus*, etc.) are highly resistant to the action of copper sulphate, but are easily killed when chlorine is applied first and copper sulphate later. The members of the blue green algae (e.g. *Oscillatoria, Anabaenopsis*) however, are very sensitive to copper. The diatom *Melosira* which occurs as a dominant form in many clear water tanks and wells on the plains and in some of the Ooty reservoirs was killed by a relatively small dose of (0.3 p.p.m.) copper sulphate even when present in enormous quantities.

Resistance to filtration.

The influence of the algal plankton present in the Stanley reservoir at Mettur on the method of water purification employed there was also studied. The interesting observation was made that in June, the plankton of the Stanley reservoir consisted of a very rich growth of *Mougeotia* which was little, if at all, affected by the addition of alum to the water. This alga caused a considerable reduction in the duration of filter runs.

Nitrogen cycle.

Studies on the regeneration of nitrogen compounds in the course of decomposition of the dead plankton were continued ; they indicated that a complete mineralisation of the nitrogen compounds took place in from 60 to 80 days in the reservoir.

The relation between the organic matter content of water and the algal plankton was studied with particular reference to the amount of organic matter contributed by the plankton algae to the water. It was observed that while *Oscillatoria* and *Arthrospira* contributed relatively larger amounts of oxidizable organic matter, *Microcystis* yielded very much smaller amounts, bulk for bulk.

Technique of enumeration.

Comparative studies on the technique of enumeration of the algae in water, the assay of the total volume of suspended matter in water and the cultivation of algal species in pure culture were continued during the year and important observations have been recorded.

A study of the results obtained with the Godavari river shows that a very well marked seasonal variation occurs in the phytoplankton of the river water, and in the attached marginal forms, found in the alum sedimentation basins and that the chief factors that influence these algal flora are the intensity of the flood, the velocity of the water, its turbidity and mineral constituents and also the temperature.

44. Enquiry into the isolation of the different constituents of snake venoms with a view to determining their chemical nature under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

Effect of crude venom and neurotoxins on the respiration of tissue cells.

The study of the effect of snake venom on the respiration of normal tissues has been continued. It has been reported previously that crude venoms of B. cerelus, Cobra, Russell's viper and B. fasciatus inhibit the respiration of brain cells of pigeons to a very marked extent. The effect of venoms of Cobra, Russell's viper and Banded Krait on the respiration of the liver cells of the pigeons has been recently investigated. It has been found that Cobra venom has got the greatest inhibiting action and that at 35°C, 0.03 per cent and 0.01 per cent solutions of Cobra venom reduce the oxygen intake of the liver cells to nearly one-half and one-third respectively of the normal value. It was thought that since the neurotoxins are responsible for the paralysis of the nerves, their effect on the respiration of the brain cells would be the greatest. Experiments have therefore been carried out with the pure neurotoxins which have been isolated by us from the crude venoms of Cobra, Russell's viper and Banded Krait. It is found to our surprise, that the purified neurotoxins have not the slightest inhibiting action on the respiration of the brain cells. The question, therefore, arises which of the various vital biochemical reactions occuring in the nerve cells, are inhibited or suppressed by the neurotoxins to bring about the paralysis of the nerves. Further work on this line is being continued.

Effect of snake venom on glycolysis in the tissues.

Another important chemical reaction that occurs in normal cells is the conversion of glucose into lactic acid. This process does not require oxygen and may be represented by the equation—

$C_6H_{12}O_6$ (glucose) = $2C_3H_6O_3$ (lactic acid).

The effect of snake venoms on this reaction in the brain cells of pigeons has been investigated. The experiments were carried out in an atmosphere of nitrogen and emulsions of brain cells of pigeons in Ringer solution containing

0.2 per cent glucose were used. It has been observed that the crude venoms of Cobra, Russell's viper, B. cerelus and B. fasciatus, reduce the rate of glycolysis to a marked extent, the effect being the greatest with Cobra venom and the least with Russell's viper venom. Experiments with the purified neurotoxins isolated from the venoms of the snakes mentioned above, show that they do not reduce the rate of glycolysis. It is evident, therefore, that crude venoms of Cobra, Russell's viper, etc., contain an inhibitor of glycolysis and respiration and this inhibitor of glycolysis and respiration is not the neurotoxin.

ISOLATION OF THE INHIBITOR OF GLYCOLYSIS FROM CRUDE COBRA VENOM.

The separation of the inhibitor of glycolysis from the crude venom of Cobra was attempted by us. It has been observed that when an one per cent solution of crude Cobra venom is treated with anhydrous magnesium sulphate and the concentration of the latter is brought to 40 per cent, a precipitate is formed which contains the whole of the glycolytic inhibitor mixed with about 10 to 15 per cent neurotoxin. If the precipitate is redissolved in distilled water and the solution saturated with sodium chloride a precipitate is again formed. This contains only about 5 per cent of the neurotoxin and the full amount of the inhibitor of glycolysis.

THE CRYSTALLIZATION OF HAEMOLYSIN FROM COBRA AND BUNGARUS FASCIATUS VENOMS.

It has been previously reported by us that the haemolysin contained in the venoms of Cobra, Banded Krait etc. is an enzyme. Only recently we have succeeded in obtaining this enzyme in a crystalline condition by a complicated method of fractional precipitation with salts like sodium chloride and ammonium sulphate and by adsorption with Alumina C. The difficult nature of this problem will be realised from the fact that out of hundreds of enzymes only about a dozen has been crystallised so far. The isoelectric point of this crystalline haemolysin has been determined and has been found to lie between pH 8.56 and pH 8.61. The molecular weight of the enzyme has been found to be 31,800 as determined from diffusion coefficient measurements. From an analysis it has been found that the crystalline haemolysin contains:—Arginine 18.94 per cent, Histidine 11.25 per cent, Lysine 5.23 per cent, Cysteine 11.6 per cent and Methionine 3.62 per cent.

45. Enquiry on tetanus under Dr. B. B. Yodh, Lamington Road, Bombay.

The study on the effect of intraperitoneal injection of serum obtained from patients suffering from tetanus, in white mice and guinea-pigs was continued during this period.

Sera were obtained from 40 cases and injected in 39 guinea-pigs and 41 mice. Nineteen guinea-pigs and 21 white mice were used as controls. The behaviour of the animals in the following manner was considered positive and any other mode of reaction negative.

1. Guinea-pigs: (*a*) spasmodic movement of the head which is thrown forwards and upwards; (*b*) sitting quietly with both the forelimbs fully raised and stretching up or out one of the limbs and bringing it down suddenly; (*c*) twisting the body laterally or the neck in a screw-like fashion; (*d*) remaining inactive with all the forelimbs fully erect and moving slowly with a reeling gait. Any one or a combination of these.

2. White mice: (*a*) stretching the hind limbs backwards and outwards; (*b*) twisting the body laterally; (*c*) inactive with all the limbs or the hind limbs fully erect. Any one or a combination of these.

Applying these criteria to the animals used it was found that of the 41 test mice, 14 showed positive reaction and survived; 18 showed the positive reaction and died; 7 did not react in the typical way and survived for 10 days. Of these last 6 were injected with sera obtained from patients before any A. T. S. was given to them, 5 of these cases being mild and 1 moderately severe. Two animals did not react in the typical way but died within 20 hours, although the sera injected were obtained before any A. T. S. was given to the patients who were mild cases of tetanus when the blood was withdrawn.

Of the 21 control mice, none showed the typical reaction, and 18 survived for more than 10 days.

Of the 39 test guinea-pigs, 36 showed the positive reaction, 22 of which survived the 10-day period of observation; the rest died within that period, but mostly within first 24 hours. Three did not react in the typical way and were alive 10 days later. In these cases blood was obtained from 2 severe and moderately severe cases of tetanus before any A.T.S. was given to them.

Of the 19 controls, none showed the typical reaction and 17 of these survived for more than 10 days.

These observations are in complete accord with the result obtained in the last financial year, which is as follows:—

Mice:

Reaction	positive and alive	54
,,	negative ,, ,, ...	39
,,	positive ,, died	37
,,	negative ,, ,, ...	6
		136

Guinea-pigs:

Reaction	positive and alive	26
,,	,, ,, died	15
,,	negative ,, alive	17
,,	,, ,, died	3
		61

These reactions as seen in the white mice and the guinea-pigs are so typical that they can be considered diagnostic of tetanus. These animal inoculation tests are of particular value when the diagnosis of tetanus is in doubt, as the result can be obtained within an hour or two, and the specific treatment can be immediately instituted if the result is positive.

The recording of the spasms on the kymograph machine was continued, and 102 records were obtained from 47 patients:

1	record	each from	15 patients.
2	records	from	19 patients.
3	,,	,,	8 ,,
4	,,	,,	2 ,,
5	,,	,,	2 ,,
7	,,	,,	1 ,,

Frequency and severity :—

22 records show very severe and frequent* spasms.
5 ,, ,, very severe and less frequent ** spasms.
26 ,, ,, moderately severe and frequent ** spasms.
15 ,, ,, moderately severe and less frequent spasms.
12 ,, ,, mild and frequent spasms.
20 ,, ,, mild and less frequent spasms.

*frequent=more than 6 spasms in 15 minutes.
**less frequent=less than 6 spasms in 15 minutes.

Sedatives :—

11 records with no sedatives.
2 ,, ,, Mist. Chloral and bromide.
51 ,, ,, Paraldehyde per rectum.
11 ,, ,, Somnifein injection intramuscularly.
20 ,, ,, Mist. Chloral and bromide, and paraldehyde per rectum.
1 ,, ,, Mist. Chloral bromide and Somnifein inj.
3 ,, ,, Paraldehyde per rectum and Somnifein inj.
3 ,, ,, Mist. Chloral bromide, paraldehyde per rectum Somnifein inj., Seconal pulvules etc.

In the majority of cases the sedatives were administered not more than 3-4 hours before the recording was started. Paraldehyde per rectum was given during the recording of 36 graphs, and Somnifein inj. during the recording of 2 graphs.

In 24 records only out of a total of 102, is there evidence of the spasms diminishing in frequency, and that too, when the spasms were mild to begin with. In most of these cases paraldehyde per rectum was the sedative used, and on 17 occasions it was administered during the actual recording of the spasms. No change was seen when the spasms recorded were severe from the beginning.

In three instances where the patients died shortly after the recording was stopped, the records show progressive increase in the frequency of the spasms.

46. Enquiry into vaccine prophylaxis of bacillary dysentery under the Director, Central Research Institute, Kasauli.

An enquiry into the problem of the preparation of a prophylactic vaccine against bacillary dysentery was conducted by Major W. Leonard Forsyth. The investigation concerned the Flexner and Shiga groups of organisms and each was examined in turn.

Flexner strains recently isolated and furnished by the Officer Commanding, Enteric Depot, were considered from the antigenic point of view and the detail of work done subscribed only to this issue.

The toxicity of the most prevalent strains was first determined and most of the work that followed was done with a selected V strain.

These V strains *inter se* showed minor degrees of difference of toxicity varying from an a.l.d. of 0.16 mg. to 0.32 mg. being the dose required to kill the majority of 20 gramme mice in 48 hours. The closely related group strains W and Z and also two of Boyd's specific strains were found to possess similar toxicity to mice. Comparative figures for B. Shiga and a Vi strain

of B. typhosus were 0.04 mg. and 0.08 mg. respectively. The toxic dose remained thermostable on heating at 60°C. for 1 hour.

Detoxication of V strains by merthiolate and formaldehyde in various strengths was compared. Formalin 2% for 3 weeks appeared to be the optimum amount, detoxicating five times and raising the a.l.d. to 0.75 mg. The virulence dose of live 18 hour culture averaged 360×10^6 being the dose required to kill 50 per cent of 20 gramme mice within 7 days. Although the a.l.d. of a few strains was 250×10^6 nothing approaching 3.50×10^6, given in an American journal, was ever obtained.'

These organisms are of somewhat low virulence but this can be enhanced by Rake's mucin technique used in subsequent immunological determinations. A comparative figure of 10 to 40×10^6 for virulence for B. Shiga was determined.

The selection of a suitable antigen was made after trial of the following along orthodox lines:—(1) lipoid extract (acetone). (2) remaining fraction "acetone cells". (3) 'bleach' detoxicated cells. (4) supernatant washings (ecto-antigen) and (5) formalin 2 per cent detoxicated whole cells.

Several of these proved good agglutinogenic agents in rabbits but the best immunogen in active and passive experiments in mice was the formalin detoxicated antigen.

In active immunity, in repeated experiments, mice were protected against 100 M.L.D.'s in mucin after subcutaneous vaccine doses amounting to 1/10th a.l.d. of formalin-detoxicated whole cells, while 0.5 c.c. of serum of rabbit immunised by 1.5 mgs. in three weeks protected against 100 M.L.D. in mucin and against 5 M.L.D.'s intraperitoneal inoculum in straight tests.

Immunization of monkeys by 0.5 mg. followed by 1 mg. and succeeded by feeding with 4 mgs. of moist Flexner culture or stool mucus resulted in infection of 7/20 non-immunised animals with two deaths while 20/20 immunised remained unaffected—statistically significant and reported *in extenso* elsewhere.

As a result of the foregoing, a vaccine is proposed for trial consisting of

Flexner V 0.5 mg.
,, W 0.25 mg.
,, Z 0.25 mg.

1 mg. per c.c. or 3146×10^6 per c.c.

This vaccine is the equivalent of No. 7 opacity Brown's tube and has been inoculated in doses of 0.5 c.c. and 1 c.c. to twenty individuals. The local reaction is mild and general reaction without malaise, and much less than that produced by T. A. B. vaccine. Resulting agglutinin titres 1/50 to 1/500 were obtained.

Shiga infection.

The investigation concerning B. Shiga followed similar lines. Five recently isolated strains were examined. Toxicity tests using 18 hours culture in 0.5 per cent carbolic, killed the majority of 20 gramme mice in 7 days, with doses ranging from 0.04 to 0.08 mg.

The average lethal virulence dose of live 18 hour culture in broth was 0.1 c.c. the majority of mice being killed in 7 days. The equivalent viable counts were 10 to 40×10^6 per a.l.d.

Detoxication—Choice lay between formalin 2 per cent and alcohol 75 per cent after eliminating other possible chemical agents. Mice survived 0.6

mg. dose of formalin detoxicated Shiga and only 0.4 mg. of alcohol detoxicated substance. The alcohol vaccine, however, produced a higher agglutinin titre in rabbits and the serum of these animals protected mice better than that produced by the formalin prepared vaccine.

Titres of 1/2500 to 1/5000 were obtained in rabbits by intravenous inoculation of 1.5 mgs. without loss of weight, enterotropic followed or neurotoxic disturbance. The dose for the monkey of 0.5 mg. followed in 4 days by 1 mg. seemed satisfactory; not too toxic, and producing agglutinin titre of 1/50 to 1/500.

Intradermal injection in guinea-pigs resulted in a minimal degree of inflammation without necrosis. A Shiga vaccine, alcohol-detoxicated preserved in 0.5 per cent carbolic is suggested for prophylaxis in man in doses of 0.43 mg. and 0.86 mg. or 0.5 and 1 c.c. of No. 6 opacity Brown's tube (2777 million per c.c.).

The detail of preparation of Flexner and Shiga vaccines will be found elsewhere. As opportunity offers both vaccines are being tried to determine local and general reaction and, where possible, the assessment of protective antibody.

It is considered that principles have been arrived at which will indicate suitable methods of detoxication of Flexner and Shiga vaccines without reducing antigenic value. Levels of dosage are indicated and a field trial of vaccine prepared on these lines is recommended.

Definite protective value is considered to have been shown in monkeys against infections by the natural oral route.

47. Immunological studies in allergic states under the Imperial Serologist, Calcutta.

This enquiry was initiated to study the various allergic states with special reference to the skin lesions such as leucoderma, psoriasis and urticaria. Three leucoderma cases and two cases of hyperpigmentation of skin have been treated with auto-urinary proteose. The hyper-sensitiveness of the patient was first tested by dermal reaction using proteose solutions varying from 1 in 1000 to 1 in 100,000 dilution. A control test with normal saline was also done in every case. The dilution in which the least reaction was observable was used for the first injection. The dose was gradually increased. The injections were given once a week hypodermically. Each patient received a course of twenty injections. Two patients required two fresh courses of treatment. In all the cases the skin lesions improved but before a complete cure could be established in all the patients, with the exception of two, failed to return for the treatment.

48. Haematological and immunological studies on normal blood under the Imperial Serologist, Calcutta.

(Introduction :—The Officer-in-charge of this enquiry, Lt.-Colonel S. D. S. Greval, I.M.S., was called for military duties in the middle of July 1941 and since then the enquiry is being continued under Dr. S. N. Chandra).

In connection with the work of the Blood Bank it was thought desirable that a systematic study should be undertaken on normal blood both from haematological and immunological aspects and certain Indian standards of normality ought to be fixed up. The following investigations were carried on :—

Haematological studies on normal blood.

(*a*) *Haemoglobin percentage of normal blood*—A total number of 250 samples of blood were examined and it was observed that 90% of the samples had a haemoglobin of 80 to 100%. This result compared very favourably with the average cell volume of these samples. The samples were mostly collected from the various jails in Calcutta.

(*b*) *Determination of cell volume of normal blood*—The cell volume was determined by the standard method of Wintrobe's tubes. Oxalated blood was used for this purpose. A total number of 75 samples were tested. On an average the samples contained 42% cells and 58% of plasma. This also compared favourably with the findings of previous workers.

(*c*) *Fragility of red blood cells,* fresh and stored—This work was undertaken to find out the period for which red blood cells could be stored in a refrigerator at a temperature of 4 to 6°C without undergoing haemolysis. Each sample of blood was tested twice, once as fresh and the other after storage, in three different concentrations of saline. It was observed that there was no appreciable increase in the fragility of the red cells upto the 12th day of storage. After this period the fragility of the cells increased gradually till on the 15th day 20% of the cells were haemolysed in normal saline.

(*d*) *Specific gravity of normal blood*—So far as we know no systematic work has been done to determine the specific gravity of blood amongst the Indians excepting that of Sir Leonard Rogers, in connection with the hypertonic saline treatment of cholera patients. In this work mixtures of glycerine and water of varying specific gravities between 1,054 and 1,064 were used. Mixtures of chloroform and benzene were found more suitable for this purpose as the dispersion of the drop of blood was minimum and hence these experiments were done in this medium. The special advantage of this mixture is that the drop of blood either sinks or floats as a globule for a longer time than in the medium used by Sir Leonard Rogers and hence the results can be read more accurately. To guard against experimental errors due to volatilisation of this medium it is essential that the experiment should be conducted in a cool room like the one in the School of Tropical Medicine. A comparative series was also done with the glycerine water mixtures as well and the two methods were very closely comparable. Two hundred and forty samples of blood were examined and it was found that 50% of these had a specific gravity of over 1060.

Immunological studies on normal blood.

(*a*) *Opsonins in normal blood*—The technique followed in this experiment was very simple. A measured drop of whole blood was placed on a clean slide containing a drop of 0.6% sodium citrate solution. To this was added a drop of blood to be examined and a drop of saline suspension of staphylococcus citreus (500×10^6 per c.c.). These were thoroughly mixed on the slide and kept in a moist chamber at room temperature for half an hour. A drop of this was spread out on a clean slide, and was stained with Leishman. The number of cocci ingested by each polymorphoneuclear leucocyte was counted. Average was taken from a count of 100 such leucocytes. One hundred and forty samples of blood were examined. It was found that each leucocyte had ingested on an average of 5 to 8 cocci.

(*b*) *Opsonins in certain morbid state*—The blood of 60 diabetics was tested for this purpose with the technique described above. In this case the average rate of ingestion of cocci was 1 to 3 per leucocyte. No relationship could be

established between the blood sugar content and the opsonic power excepting that people with high blood sugar showed a decreased opsonin content in their blood.

(*c*) *Titration of complement of fresh human blood*—This was a preliminary experiment to find out whether there was any rise or fall in complement with a view to gauge the effect of treatment on patients in health and disease. One hundred and twentythree samples of fresh human sera were examined for their complement titre by the usual standard method. It was found that the average complement titre was 1 in 10.

Blood Bank.

This enquiry actively associated with the working of the Blood Bank started in October 1939 in Calcutta in conjunction with the Bengal Provincial Branch of the Indian Red Cross Society under the Imperial Serologist. A summary of the work carried out in connection with this from the beginning of this financial year upto October 1941 is given below:—

Number of persons examined for their fitness and suitability to serve as blood donors in future	207
Number of donors bled for transfusion and storage	130
Number of bloods supplied to the various hospitals in Calcutta, free and paying, on demand	76
Plasma was separated from time expired blood and was disposed of as follows:—	
(*i*) to the cholera bacteriological enquiry at the School of Tropical Medicine	3,500 c.c.
(**ii*) for experiments on concentration and drying of plasma	2,700 ,,
(*iii*) balance in storage up-to-date	600 ,,

(*this includes a litre of plasma supplied to Major S. S. Bhatnagar, I.M.S., for drying experiment and 135 c.c. of plasma given to Dr. L. E. Napier for animal experiments).

Concentration of plasma.

A few experiments were done on the concentration of plasma. The method used was as follows: Plasma was removed from time expired blood (10 to 15 days) and made safe for transfusion regardless of the group by reducing its isohaemagglutinin titre to a safe level (by mixture or absorption). Sterile cellophane bags were charged with plasma and left in a room close to a fan. Concentration was determined by weighing the bags. A small amount of plasma was concentrated by this method. Experiment under vacuum condition was carried on with limited quantity but was given up later as the production was insufficient under the present laboratory arrangements for mass production.

49. Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under Lt.-Col. S. N. Hayes, I.M.S., Medical Superintendent, Lady Willingdon Hospital, Lahore.

The enquiry was started for the purpose of investigating methods of drying plasma—suitable for Indian conditions. No knowledge was available in India on the subject. The following methods were investigated and considered.

(1) *Greaves and Adair*—This method was considered too complicated and there would be difficulty in obtaining the necessary apparatus. The product produced in England was, according to the literature, satisfactory for Western conditions. We decided not to consider this method owing to the difficulty in obtaining the requisite apparatus.

(2) *Edwards, Kaye and Davie*—This method was simple and cheap. Apparatus was available and the product was concentrated in a small bulk. Theoretically the product and its preparations were ideal for Indian conditions. The method was fully investigated—(see later).

(3) *Cryochem method of Flosdorf and Mudd* was investigated and reported on.

(4) *Desivac process* was considered and reported on.

(5) *Adtevac process* of Hill and Pfeiffer was investigated and reported on.

Method of Edwards, Kaye and Davie.

Plasma for serum was dried in vacuo at 37° by means of a simple apparatus. Nine batches were dried, using a closed circuit method.

All batches were sterile. The moisture content of each batch was estimated and showed an average of 13.1%. This was theoretically excessively high, and could not be reduced below 7.97% which was also too high.

The batches were kept for a period of three months at 2°-5°C and room temperature and tested for solubility. Those kept at low temperatures were more soluble than those at room temperature, (up to 45°C). The degree of solubility decreased with the time of keeping and the increase of temperature.

Transfusions of the dissolved product showed that with the freshly prepared product the reactions were nil or not abnormal, but that reactions increased with the duration of storage and the increase of temperature.

Taking into account the high percentage of moisture—laboratory, clinical and chemical experiments—we concluded that this simple method could not produce a product that was suitable for Indian conditions.

Cryochem method of Flosdorf and Mudd.

Laboratory experiments were conducted using calcium sulphate anhydrite prepared from indigenous gypsum as the dessicant. The resulting product was superior to that obtained by the method of Edwards, being light, porous and easily soluble. The minimum percentage of miosture obtained was 1.6%. We decided that the method was unsuitable for large scale production as the preparation of the dessicant done in the required quantities would entail a separate organisation and increase expenses of manufacture.

Desivac process.

This process was considered and as it was already established as a commercial process, and in view of its simplicity—the apparatus consisting only of a dessicatory chamber in which the frozen plasma serum was placed and to which a specially designed vacuum pump was attached—it was recommended that Government should purchase two Desivac plants. Later it was advised that only one plant should be purchased, pending investigation of a new process (the Adtevac) and in view of the large amount of work that was being conducted, which might result in a simpler method being evolved.

ADTEVAC PROCESS.

Since June we have investigated the above from the laboratory point of view. The process has many advantages i.e. its working cycle is short, 6 to 12 hours (as against 80 hours with Desivac). The dessicant used is silica gel which can adsorb 30% of its own wieght of moisture and lasts indefinitely. The final product contains a very low percentage of moisture *i.e.* 0.03%.

With the equipment and information at our disposal, we have not been able to reduce the moisture content below 1.6% but the method is simple and attractive and has many advantages. The authors unfortunately are unable to give us additional technical details desired, and which have been omitted from their descriptive article—owing to the method having been put on the secret list by the U. S. A. Government. The method is now commercialised and we have recommended that a 10 litre plant be purchased.

50. Enquiry into the pathology and pathogenesis of splenomegaly under Dr. D. Govinda Reddi at the Medical College, Madras.

An examination was made of the autopsy protocols of Government General Hospital, Madras, for ten years from 1931-41. Out of 750 cases during this period, 280 showed enlargement of spleen, taking 7 ounces and above as abnormal. Out of 280 splenomegalies only two belonged to the Banti's syndrome, having histopathological features as described by Banti. Similar changes were noticed in two cases out of 30 splenomegalies due to chronic venous congestion in cardiac failure. On the other hand in 11 cases of splenomegalies associated with Cirrhosis of liver, none of them showed any Gandy Gamna nodules. A paper is being prepared for publication shortly.

Haematological study of cases diagnosed as Banti's syndrome in the Medical wards of Government General Hospital is undertaken. So far there were only 3 cases.

Examination of the vasculature of the spleen after perfusion: work is being carried out in this line at present.

51. Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

During the course of the work for the last seven months sixty cases of wound infections were studied drawn from the following sources:—

	Number of cases.
Surgical cases	12
Casualty wounds	28
Post-mortem material	7
Soil and manure	7
Human and guinea-pig stools ..	3
Other sources	3
Total number ..	60

It will be seen from the table that a variety of material has been utilized. Material collected from animal sources was used as a basis of comparative study.

The following strains have been isolated.

Clostridium	Welchii ..	.. 8
,,	Septique	.. 4
,,	Histolyticum ..	.. 1
,,	Tetani ..	.. 4
,,	Botulinum ..	.. 1
,,	Sphenoides ..	.. 2
,,	Sporogenes ..	.. 17
,,	Unidentified ..	.. 7
Total number of strains		.. 44

Every strain that has been isolated was injected into mice to test their pathogenicity. All the pathogenic strains have been obtained from definite cases of gas gangrene. Of the eight cases of gas gangrene Clostridium Welchii was isolated in five cases, singly or in combination with other pathogenic or non-pathogenic anaerobes. In the remaining three Clostridium Septique was the causal organism and it was associated with Clostridium Sporogenes in two cases. In two cases Clostridium Welchii and Septique occurred in combination.

From cases of deep wounds without evidence of gas gangrene admitted to the Casualty department no pathogenic anaerobes could be isolated. A few of them yielded cultures of non-pathogenic anaerobes of the type of Clostridium Sporogenes. From a study of twentyeight cases of road-side deep wounds it appears that infection with anaerobic pathogenic organisms is uncommon in civil practice.

Clostridium Welchii.

As stated before this anaerobe has been the principal organism concerned in the causation of gas gangrene. The invasiveness of this organism varies with the various strains. Toxins isolated from the organisms have in some cases killed the animals while in others they failed to do so. This might perhaps be due to a difference in the capacity on the part of the organism to produce toxins. Attempts are being made to find out the factors responsible for the variation in the virulence of the toxins produced by Clostridium Welchii.

Animal passage through a susceptible animal such as the pigeons was a method adopted to increase the virulence of the organism. Various media have been described as being suitable for producing maximum content of the toxin in its virulent state and these media are under trial.

This work is of importance to determine whether the local strains are toxigenic since this will have a bearing in the treatment of gas gangrene as to whether an antitoxic or an antibacterial serum or a mixture of both is essential for therapeutic use.

52. Haemotological survey of poor class families in and around Lucknow under Dr. V. S. Mangalik at the King George Medical College, Lucknow.

The work was started in May 1941 to find out the incidence of anaemia in poor class people living in and around Lucknow. It was observed that the poor class people in this part of the country showed an apparent low incidence of anaemia, and this was in spite of their low economic income and consequent

intake of a diet which was likely to be deficient, apart from other things, in essential haemopoitic substances.

Methods.

A record of the age, income, profession and occupation, number of dependents on the wage-earner has been kept and in case of women, the number of children borne, and age of the last child. Haemoglobin estimations have been made from capillary blood. Hellige Haemometer has been used all through—the same instrument has been used for all cases, and the technique as laid down by Napier & Das Gupta (1935) has been followed. Results have been expressed in grams per 100 c.c. of blood (100 per cent=13.75 grams %).

Locality.

This survey has so far been confined to poor people, residing in an area in Lucknow called Hyderabad and Badshahbagh, where these people are employed as domestic servants, chaukidars, malis or garden coolies etc., mostly in the University Colleges and Hostels, and with the members of the University staff. Some earn their livelihood by working as washermen, barbers etc. A few hundred cases were investigated at two suburban villages situated about 4 or 5 miles from Lucknow.

Subjects.

In all, 1,287 individuals have been examined—1,064 males, and 223 females. No one with a monthly income of over Rs. 20/- p.m. has been included in this series. Average income of the wage-earner has been worked out to Rs. 8/- per month. The average number of dependents, which the wage-earner has to feed on this income, in addition to his ownself, has been 2. The individuals concerned, it will be seen, thus constitute a cross section of the very poor class of people of this place.

The subjects never presented themselves to hospitals or dispensaries with any illness or complaint. They were usually approached at their homes or fields. They considered themselves in such good health that it has been a constant source of difficulty to persuade them to have their blood examined.

Cases of all age groups have been included—and all members of a family were examined as far as possible. Number of cases in certain age groups is low, and so is the number of females—223 in a total of 1,287.

Standards of normal haemoglobin.

Most of the standard haematological data available for Indians relates to middle class men and women, not comparable economically to the population under survey. Judging from all available data, mean haemoglobin range lies approximately between 15 and 16 grams % for males and between 12.5 and 13.5 grams % for females. The only standards available for poor class people, whose economic status could be compared to subjects under survey, are for Assam and Cachar coolies, worked by Napier & Billimoria and Napier and Majumdar. Mean haemoglobin of this class comes to about 12 grams for males and 10.5 for females. We have, however, drawn a line at 10.5 grams to distinguish anaemic from normal people. Cases below this figure have been classed as anaemic.

Diets.

Only a qualitative survey of the diets has been done. It was found that most people take 2 meals a day and the meal consists of chapaties and Dal or Dal and Rice. Usual "dal" consumed is Arhar. Vegetables (mostly green sags) are eaten once a week. It is rare for them to have milk or fruits. It was revealed that a vast majority of these families use *iron utensils* in the kitchen either to knead flour for chapaties or for cooking vegetables.

Results.

	Males.	Females.
1. Total number of cases examined ..	1,064	223
2. Persons having haemoglobin over 10.5 grams % and classed as "normal" ..	1,031	200
3. Persons having haemoglobin less than 10.5 grams % and classed as "anaemic" ..	33	23
4. Percentage of anaemic cases ..	3.1%	10.3%

Haemoglobin average of "normal" cases on the basis of 10 years age groups could be represented as follows:—

Age groups.	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80
MALES.								
Number of cases ..	222	359	237	132	54	26	3	2
Mean haemoglobin in grams %	13.2	14.2	15.0	15.0	15.2	14.3	13.4	15.6

Mean haemoglobin for all age groups=14.3 grams %.

FEMALES.								
Number of cases ..	60	39	49	27	17	7	1	0
Mean haemoglobin in grams % ..	12.8	12.6	12.6	12.2	13.5	13.0	11.7	..

Mean haemoglobin for all age groups=12.7 grams %.

From the point of haemoglobin-range, the subjects can be grouped as follows:—

Group I. Haemoglobin *above* 14.0 grams %.
Group II. Haemoglobin *between* 10.5 & 14.0 grams %.
Group III. Haemoglobin *below* 10.5 grams %.

Distribution of subjects into these haemoglobin groups was as follows:—

	Total.	Group I.	Group II.	Group III.
Males	1,064	608 (57.1%)	423 (39.7%)	33 (3.1%)
Females ..	223	37 (16.5%)	163 (73.0%)	23 (10.3%)

On the whole, judging from above data, and comparison with normal haemotological values in middle class people and in Assam coolies, the haemoglobin values of poor class people in this area are surprisingly satisfactory. They do not reflect an inadequate intake of iron, though the diets are apparently quite poor. The incidence of anaemia (haemoglobin of less than 10.5 grams %) also surprisingly low—3.6% for males and 11.6 % for females.

The importance and value of almost universal use of iron utensils for cooking in this class of people needs to be more fully worked out. When even simple boiling of milk in iron utensils transfers appreciable quantities of iron to milk (enough to turn a cup of tea black), it seems all the more likely that larger quantities of iron will be transferred to food substances which are subjected to prolonged cooking in iron utensils. A look at vegetables cooked in iron karahi (sauce-pan) will convince one of the validity of this observation.

This observation bears relation with this survey and hypothesis is advanced that, in spite of poor diets due to poverty, the population shows comparatively good haemoglobin values. This is perhpas due to intake of adequate amounts of iron from the utensils during the cooking of food. This aspect of the problem needs more thorough investigation. This hypothesis has been tentatively advanced in the hope that more work will be done in this direction to prove or disprove the hypothesis. The work is still in progress.

53. Leptospirosis enquiry under Dr. M. N. Lahiri at the Haffkine Institute, Bombay.

This enquiry was commenced in the beginning of the year 1940, the objects being to detect the leptospiral nature of the disease and to carry out a complete study in regard to its incidence, epidemiology and other standpoints. In this country the existence of the disease has now been definitely established in Calcutta. It has been emphasised that certainty regarding diagnosis must be attained bacteriologically without which the diagnosis on clinical grounds alone cannot be accepted as reliable.

Culture medium.

Vervoort's media according to Schuffner's formula was used throughout for the culture and the maintenance of the strains of leptospira. But certain modifications had to be introduced to standardise the media to suit the local condition. The culture media is now prepared in small bulk as follows:—

To 155 c.c. of distilled water 0.14 gramme of Bactopeptone is added. To this Ringer's solution 30 c.c. and Sorensen's double phosphate buffer mixture pH 7.2 are added. The mixture is boiled and allowed to cool; filtered through Kiselguhr covered filter paper to get rid of the fine particles (usually ½% Kiselguhr suspension forms a good film over the filter paper) and then the reaction is checked. The solution is sterilized in autoclave for 20 minutes. Finally to complete the medium fresh sterile rabbit serum is added to a concentration of 10% and heated at 56°C for 30 minutes to inactivate the serum. The pH of the finished medium remains between 7.2 and 7.4. The medium is tubed in sterile clean test tubes in 6 c.c. quantities and at the same time tested for sterility.

Leptospiral infection in rats.

This study was pursued with a view to demonstrating the infection and identifying the spirochaete with reference to its serological classification. It has a bearing on the epidemiology of the disease. The following methods of investigation were used :—(*i*) direct examination of the kidney cortex emulsion by dark-ground illumination (*ii*) inoculation into animals, (*iii*) culture of the kidney cortex (*iv*) serological testing of the rats sera for the presence of agglutinins and lysins. Of the 125 rats trapped in a particular congested area of the City and examined by the above methods leptospirae were detected in eleven of them by the direct method, and five by animal inoculations two of which were of course negative by the direct method. Two other animals gave evidence of infection serologically. Thus 15 or 12 per cent of the rats were found to be vectors of *leptospira icterohaemorrhagiae.* The results of the culture of the kidney cortex were far from satisfactory on account of an inter-current infection in most of the rats due to Bact. Pseudo-asisticum. The observations indicated that no single method of investigation is foolproof and therefore the importance of the combination of various methods is stressed. The rats found to be infected all belonged to the species *Rattus norvegicus* except one which was of the species *Rattus rattus* and whose serum showed anti-leptospiral agglutinins up to a titre 1 : 640. The five strains of leptospira isolated from the rats all reacted up to the full titre with anti-leptospiral serum (Classical) prepared here.

Transmission of leptospiral infection from mother to the foetus.

Definite observations as to how the infection of pathogenic leptospirae is maintained in the rat population are lacking. In course of the studies on the incidence of infection in rats a carrier pregnant rat was trapped. Serologically identical strains of leptospira were isolated from the mother as well as the foetuses. This observation presented evidence in support of natural infection in the rat through placenta. Accordingly it is suggested that the infection in rats with *L. icterohaemorrhagiae* is likely to be maintained through the placental route.

Human cases of the disease.

To enlist the co-operation of the hospitals a memorandum on leptospirosis was prepared. It has been decided to tackle all cases of acute jaundice with fever and this seemed to be the best line of approach for the detection of the disease. During the past six months eight genuine cases of the disease have been recognised. The number of reported cases does not present a true picture of the actual incidence. The diagnosis have been confirmed either by the isolation of the organism or by serological tests. Opportunity to carry out cultural examination of the blood did not present itself so far as most of the cases were notified at the time when the infective period of the blood had passed.

Gradually contacts are being made with many more hospitals and members of the independent medical profession are also partly co-operating with this enquiry.

REPORTS OF ADVISORY COMMITTEES.

REPORT OF THE CHOLERA ADVISORY COMMITTEE.

The Committee met on the 15th December, 1941. The reports of the several enquiries were considered and are summarised below.

Cholera field enquiry in Bengal under Dr. S. R. Pandit.

The work of this enquiry was carried out in Calcutta during the year. In view of the reported association of the El-Tor type of vibrio in cases clinically resembling cholera in the Dutch East Indies special investigations were made as to the presence of such organisms in cholera cases. In a series of 284 cases from which vibrios were isolated none were haemolytic.

The percentage of positive isolations was 81 and at the height of the epidemic 92% were positive. The Ogawa sub-type prevailed. Non-agglutinable vibrios were not encountered in higher proportion in negative cases than in association with the agglutinable vibrios in positive cases. Direct plating gave a higher proportion of positive results than enrichment methods. A further study was made of vibrio haemolysis and it was found that strains could be divided into two groups:—(*a*) "Early haemolytic" corresponding to Greig-positive strains and (*b*) "Late haemolytic" corresponding to Greig-negative strains. On continuous incubation of cultures in contact with goat cells haemolysis was complete in a few minutes to 24 hours and was not affected by carrying out the test at 12°C or under reduced oxygen tension or by excluding oxygen, with the early haemolytic type. In the case of the late haemolytic strains haemolysis is usually partial and hardly occurs in 24 hours and does not occur at 12°C or under anaerobic conditions. Haemolysis by the "Early haemolytic" strains is specifically neutralised by anti-haemolytic serum but this effect is not produced on "Late haemolytic" strains. The haemolytic agent of the "Early haemolytic" strains is not removed by filtration through an L_3 candle while that of the late haemolytic strains is removed. The Celebes strains examined showed the same haemolytic characters as classical El-Tor strains and the Indian haemolytic waters strains.

Water examinations—During June when cholera was epidemic no *V. cholerae* was isolated from 55 samples of Hoogly water examined. An Ogawa haemolytic strain was isolated from the waters of a Jhil in the outskirts of Calcutta.

Cultural methods—The following modification of the solid medium of Wilson and Reilly gave good results in direct plating of stools:—

Papain digest agar 2.5% pH 8.8	..	..	100 c.c.
20% sodium sulphite	..	..	4.8 c.c.
Liq. bismuthi	..	..	0.16 c.c.
Alcohol absolute	..	..	0.2 c.c.
Mannose solution equivalent to	..	..	0.1 gr.

This was found superior to Aronson's medium in its inhibitory effect on other organisms and inhibited El-Tor strains and inagglutinable strains in some cases.

Improvements in isolations from stools but not from water was obtained by increasing the proportion of bismuth in the modified Wilson and Blair medium three fold to five fold.

Field cholera enquiry in Madras Presidency under Director, King Institute, Guindy.

The headquarters of this enquiry were transferred to Guindy during the year owing to the non-occurrence of cholera in the Cauvery delta which had been under study.

Laboratory studies.

Vibrio haemolysis under different conditions was investigated on a re-examination of strains collected in previous years. Consistent results were obtained in isotonic broth with 24 hours incubation. The results on super-imposed goat-blood agar followed closely those in Douglas broth. Storing plates in the ice-box for 24 hours, after a 24 hours preliminary incubation, brought out the difference between haemolytic and non-haemolytic strains clearly. Improved methods of preparing mannose from ivory nut were worked out.

Incidence of sub-type infections in the Madras Presidency.

87 strains isolated in the Northern Circars and the Ceded districts were examined. All with the exception of 1 Inaba sub-type from Guntur were non-haemolytic Ogawa sub-type. The epidemic in the Northern Circars was a continuation of that of last year which was also Ogawa sub-type.

Field investigations—Two outbreaks were investigated in difierent areas during the year. At Ampalam in the Vizagapatam District cases had ceased to occur when examinations were made but positive isolations of non-haemolytic Ogawa strains were obtained from direct contacts who remained healthy. On subsequent re-examination the vibrio could not again be recovered. A similar finding was made in another area of the same district. This confirms the existence of the healthy close-contact carrier and the short period for which the vibrio is excreted.

A re-examination of stools of individuals in a village six months after an outbreak failed to demonstrate the presence of *V. cholerae* in members of families in which cholera had occurred.

The examination of a large number of tank waters near Negapatam showed the presence of agglutinable vibrios of the Ogawa haemolytic type. The method of filtration in the field and the use of buffered KCl-boric acid saline with transmission by post to the laboratory for subsequent culture in modified Wilson and Blair medium proved successful.

Cholera bacteriological enquiry at the School of Triopcal Medicine, Calcutta under Dr. G. Panja.

The following lines of investigation were carried out up-to-date :—

(1) *Treatment of cholera by transfusion*—A few cases only were treated with human plasma owing to shortage of plasma and no opinion can be given on the value of the method. The necessity of using pyrogen--free saline in transfusion was demonstrated.

(2) *Treatment of cholera by oral administration of dyes.* Brilliant green in 1 in 5000 dilution was given by mouth in half ounce doses every 2 hours. Clinical improvement was noticed.

(3) *Isolation of V. cholerae from stools*—Successful isolation from stools was obtained by growth through an L_3 candle in peptone water at pH 9.0 with 0.08% boric acid. In many cases the growth was pure and in 87% of cases isolation was obtained.

(4) *Serological*—Studies were made on the presence of agglutinin and bacteriolysin in the sera of cholera cases. In about 91% of cholera cases the agglutinin level was commonly 1 in 20. In 63% of normal sera agglutinins reached 1 in 10 only.

(5) *Other studies*—These were carried out on (*a*) bactericidal effects of dyes, and (*b*) action of sulphanilamide compounds, (*c*) differentiation of El-Tor and cholera strains and (*d*) action of lithium choloride, etc.

ENQUIRY ON CHOLERA AT CARMICHAEL MEDICAL COLLEGE, UNDER DR. D. N. BANERJEE.

Histological examinations were carried out to determine the distribution of the cholera vibrio in the intestinal wall. The vibrio was found in the wall of the small intestine but rarely in the wall of the large intestine. Vibrios were present in the mucosa, the sub-mucosa and at the base of Lieberkuhn's follicles but only in rare instances in the sub-serous layer. Histological examination showed evidence of reaction of inflammatory nature with necrosis and desquammation of epithelium. The Peyer's patches and solitary follicles and mesenteric glands showed hyperplasia and necrosis.

Histological and chemical examinations of cholera stools, also showed evidence of inflammatory reaction.

Studies were also made on the bone-marrow and adrenal glands in cholera.

Clinical trials were carried out on the value of transfusion with concentrated saline solution (20%) in cases of collapse with high specific gravity of the blood and encouraging results were obtained.

DISCUSSION.

The reports of the enquiries were discussed and the following points specially noticed :—

(1) The observations made in India by all enquiries made so far have not shown the existence of any direct association of the El-Tor vibrio with the cholera case although strains of this type have been isolated from water in Bengal, Madras, Bihar and Sind. The El-Tor type of vibrio has not been isolated from stools of either healthy persons or of cholera cases. The studies made in the last year have shown that although haemolysis may be produced under certain conditions by vibrios which can be classified as true *V. cholerae* there are differences in the type of haemolysis as compared with that produced by El-Tor strains which enable the two to be differentiated.

The Committee are of the opinion that, in general, the investigations which have been carried out during the last 7 years have established a basis of knowledge on essential points which will be of value in practical application of preventive measures and that, for the moment, the continuation of extensive laboratory of field enquiries is of less importance than the endeavour to have the findings applied.

RECOMMENDATIONS FOR THE CONTINUANCE OF EXISTING ENQUIRIES.

(*a*) Cholera enquiry in Bengal under Dr. S. R. Pandit. It was intimated that it is not intended to continue this enquiry during 1942-43.

(*b*) The field cholera enquiry in Madras Presidency under the Director, King Institute, Guindy. After discussion, the Committee recorded their opinion that under the present circumstances the lines of enquiry proposed were not of sufficient urgency to justify the recommendation of the grant asked for.

(*c*) The cholera bacteriological enquiry at the School of Tropical Medicine, Calcutta, under Dr. G. Panja. The Committee were of opinion that the proposals for investigations put up, with the exception of those in regard to treatment of cholera, were not of sufficient urgency, and did not recommend the grant asked for. Instead, it is recommended that a grant of Rs. 4,000/- be given for the purpose of certain lines of investigation on the treatment of cholera which were proposed, *viz.* (*a*) treatment by transfusion of blood plasma, serum, etc, and (*b*) with sulphanilamide derivatives or other new compounds. For this purpose it is considered advisable that the enquiry should be under the Director, School of Tropical Medicine, Calcutta, who will be able to arrange facilities for the supply of plasma, etc., in connection with a scheme for plasma preparation which is in progress.

The items of the present budget which will be included in the sum suggested are :—

(1) Pay of bacteriologist	.. .. ..	Rs. 2,568/-
(2) Pay of laboratory attendant (grade B)	..	Rs. 461/7/-
(3) Pay of laboratory attendant (grade C)	..	Rs. 378/-
(4) Pay of sweeper	.. .. ..	Rs. 192/-
(5) Contingencies	.. .. ..	Rs. 400/9/0

(*d*) The enquiry on the pathology of cholera including cholera kidney, at the Carmichael Medical College, Calcutta, under Dr. D. N. Banerji. The committee did not recommend the continuance of the grant for this enquiry.

The Committee considered a note on the endemicity of cholera in certain parts of Madras Presidency presented by Dr. Raja. It had been noticed during the field cholera enquiry in Madras Presidency that the Tanjore District had been remarkably free from cholera during the last two years. As this area is one which had been designated as " endemic " on the basis of previous investigations covering the period 1896-1925, the Committee in 1940 had asked that a similar study should be carried out for the subsequent years. Dr. Venkatraman had furnished the figures on the subject which Dr. Raja had expanded by means of a further analysis. On a preliminary examination of the figures, Dr. Raja had expressed the opinion that the endemic status of the area was doubtful, at least in the light of recent experience, and pointed out certain possible relationships of cholera to physiographic conditions.

Before a long term policy of sanitary improvements in endemic areas can be undertaken it is necessary to have such areas strictly delimited. It is therefore considered advisable that certain detailed investigations which have recently been carried out on the cholera mortality figures for south-west Bengal should be extended to cover the rest of that province, Assam and certain parts of Madras Presidency, which had been considered by previous investigators as endemic areas. For this purpose Dr. Raja requested the allotment of the grant of Rs. 2,628/-. The type of investigation to be undertaken will be on similar lines as that in the Bengal Districts already made.

The committee recommended the grant to be given.

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 2 p.m. on 15th December, 1941, and considered the reports dealing with research work on malaria carried out during the current year and the proposals for work during the ensuing year.

The existing enquiries are listed below:

Malaria Institute of India (research section).

The Director explained that the research activities of the Institute have necessarily been greatly curtailed owing to the abnormal conditions brought about by the war. The post of Malaria Research Officer remains vacant and several of the most experienced members of the staff are serving overseas. In addition to the annual malaria courses for medical officers and engineers respectively, three special courses for military personnel have been held during the year, and the total number of those who have received training at the Delhi Field Station since the outbreak of war is 122.

Much work has been done in regard to the testing of insecticides and mosquito repellents. Pyrethrum insecticides have continued to give the best results for spray-killing adult mosquitoes. The repellent value of pyrethrum extract has also been demonstrated, as a result this now forms the chief ingredient of the anti-mosquito cream supplied to the army overseas.

Many tests have been carried out on sprayers, both for spray-killing of adult mosquitoes and for the application of larvicides. An improved hand sprayer and a knapsack oil sprayer, from which rubber diaphragms and washers have been eliminated, have been designed, and are now being supplied to the army.

Laboratory researches have been restricted to the preparation of antisera for the determination of the source of mosquito blood meals and to the study of certain unusual forms of monkey malaria parasites. Further attempts have been made to secure the transmission of *P. knowlesi* by various species of mosquitoes but without success.

Field researches in certain villages near Delhi with special reference to the spray-killing of adult mosquitoes have been continued. The antimalaria operations in Delhi have again been supervised and directed by the staff of the Institute. In Orissa, epidemiological studies have been continued in a number of villages along the shores of the Chilka Lake and in the coastal area south of it. The incrimination of *A. sundaicus* as the chief vector in this area has been confirmed, and special attention has been directed to the study of appropriate control measures against this species.

A field unit has investigated malaria conditions at Ahmedabad during the past six months, and the data there accumulated are now being analysed. *A. stephensi* was incriminated as the vector. Investigations of an outbreak of malaria in the Kurram Valley indicated that *A. stephensi* is the principal carrier in the early part of the summer, whilst *A. culicifacies* is responsible for the annual autumnal wave.

The study of mosquito bionomics especially of laboratory colonies of *Aedes aegypti* and *Anopheles stephensi* has been continued, and the routine indentification of mosquitoes and other insects of medical importance has been carried out as in former years.

In 1942-43, investigations will be carried out on the following lines, as circumstances permit:—

A. *Laboratory investigations.*

1. Testing of sprays, larvicides and spraying apparatus.
2. Testing of the effect of drugs on animal malaria.
3. Continuation of work on malaria immunity.
4. Investigations on malaria parasites of man and animals.
5. Study of mosquito bionomics.
6. Investigations regarding malaria transmission.
7. Determination of food preferences of anophelines.
8. Preparation of anti-sera for precipitin tests.

B. *Field investigations.*

(*a*) *Delhi.*

1. Study of mosquito bionomics.
2. Epidemiological studies in villages.
3. Field tests on spray-killing of adult mosquitoes.

(*b*) *North Kanara.*

1. Systematic dissections of anophelines.
2. Study of bionomics of vector species.
3. Epidemiological studies.
4. Formulation of a control programme.

MALARIA TRANSMISSION ENQUIRY.

Studies which were in progress at the end of last financial year, were completed and a few fresh experiments to determine the upper thermal limiting value for the infection of *A. culicifacies* were conducted. Some results were obtained regarding the infectivity of this species at 60° and 92°-94°F. under conditions of optimum relative humidity and at about 50 per cent relative humidity under conditions of optimum temperature.

The remainder of the time was devoted to the preparation of a full report on the work already completed with *A. culicifacies.*

This enquiry is now terminated.

CHILKA LAKE WEEDS ENQUIRY.

Salinity, which in this area has a seasonal variation, is the most important factor affecting the growth of the weeds. As there are two optima, one on each side of the peak of salinity, the weeds show two seasonal growth periods.

The depth to which light penetrates the lake water varies, being least when rivers discharge silt into it in the rains, and when the violent southern wind churns up the waters. When light penetrates least, the dominant weed becomes weak enough to be snapped by wave action, and masses of it float on the surface.

Systematic observations were made on the nature of the soil in relation to the distribution of weeds, on their perennation, seed production and germination. From the view point of malaria control, an important observation was that all the weeds contain iodine. If it can be shown that this can be extracted profitably, malaria conditions around the lake might be greatly benefited by the removal of the weeds for this purpose without cost to the government.

This enquiry is now terminated.

Protozoal parasites enquiry.

This enquiry was transferred to Shillong in September, 1941. The work was mainly directed to ascertain the vector or vectors of Plasmodium (*Plasmodium knowlesi*). Over a dozen species of mosquitoes were experimented with but without success. During these experiments valuable observations were made as to the conditions which appear to be necessary for exflagellation in *P. knowlesi*, and it was found that low temperatures with a high degree of humidity provided the optimum conditions.

Further studies on immunity were carried out. Previous experiments by this enquiry have shown that the sera of animals suffering from malaria exert a protective action. Whether these sera operate by sensitising the cells or by killing the parasites, is not known. Certain experiments to elucidate this problem were carried out but as *P. knowlesi* infection in the experimental monkeys was severe, the results were inconclusive.

The existence of *P. inui* as a natural parasite in *sinicus* monkeys at the foot of the eastern Nilgiris has already been reported. Similar infection was found to be present in monkeys captured in the western Nilgiris (Gudalur).

The budget of this enquiry has now been combined with that of the kala-azar enquiry.

Enquiry on a species of avian malaria.

Little progress has been made in the study of the schizogony cycle of *Plasmodium heroni* and the course of the disease in the host (paddy birds). This, according to the author (Dr. B. C. Basu) was due to his undertaking the preparation of the Malaria Transmission Memoir which has since been compiled.

The internal organs of a number of pond herons infected with *Plasmodium heroni* were examined for evidence of exo-erythrocytic schizogony, but without success.

The duration of schizogony of the parasite is still being investigated.

It is proposed to continue and complete the following series of observations:—

(1) Study of the exa-erythrocytic schizogony of *P. heroni* particularly in the capillaries of the brain;

(2) Duration of the schizogony cycle of *P. heroni* in the paddy birds;

(3) Working out of the complete sporogony cycle of *P. heroni* in the mosquito.

Filariasis enquiry under Dr. Sundar Rao.

A survey was carried out in 9 villages in Cachar District, where the infection is said to have been introduced in recent years from Bihar. Night blood smears from 2,445 persons showed a gross infection rate of 4.7%. Out of 115 carriers, 5 were *Mf. bancrofti* and the remainder *Mf. malavi*. The percentage showing clinical signs of the disease was 4.5

The figures representing both infection and disease rates indicate that the amount of infection has remained practically unchanged since 1934, whilst there has been a gradual increase in the incidence of clinical manifestations of the disease. A mosquito survey revealed the presence of 15 species of mosquitoes. Villages in the midst of cultivated land were found to be practically

free from infection, whilst those situated in swampy areas showed a high incidence.

A survey of Santal Parganas showed infection rates (exclusively *Mf. bancrofti*) ranging from 3 to 20%.

Transfusion experiments indicate that the life of *Mf. bancrofti* in hydrocele fluid is about 18 days.

Quinine urethane has given the best results in the treatment of hydrocele. A number of drugs were tested for the general treatment of filariasis, but only allergen and ichthyol had any appreciable effect.

The programme of work proposed for 1942-43 is :—

(1) Collection of adult specimens of *W. bancrofti* and *W. malavi* from different endemic areas.

(2) Study of the problem of periodicity of microfilaria in man.

(3) Continuation of work on filarial allergen.

(4) Studies on the treatment of filarial infection.

FILARIASIS ENQUIRY UNDER DR. T. B. MENON AND DR. B. RAMAMURTI.

The study of the development and migration of the filarial worm was carried out in a species of lizard found to be naturally infected. Embryos appeared in the peripheral blood 70-90 days after inoculation. There was little or no tissue reaction on the part of the host.

Bacterial investigations of lymph or hydrocele fluid from cases of human filariasis yielded negative results except in a small proportion of cases where organisms of mild virulence were present.

The effects of a number of drugs were tested, but the results were generally disappointing. Attempts to secure infection of animals with human filariae were unsuccessful. Attempts to prepare a vaccine from *Mf. bancrofti* were also unsuccessful.

The programme of work proposed for 1942-43 is :—

(1) Experimental studies to discover an animal susceptible to filarial infection.

(2) Study of the role of bacteria in filarial conditions.

(3) Studies on the histo-pathology of filarial lesions.

(4) Investigations regarding the treatment of filarial infection.

PHARMACOLOGICAL ENQUIRY.

(*a*) *Sulphathiazole*—The drug has little action on the cardiovascular, gastro-intestinal, genito-urinary and central nervous systems when given intravenously, but exerts a stimulant action when applied directly to the CNS. by ventricular or cisternal puncture. It is rapidly absorbed and excreted when given orally, the channel of excretion being the kidneys. It permeates the placental barrier fairly easily, and is found in measurable quantities in the CS. fluid. It is superior in the local treatment of wounds to the other sulphanilamide derivatives tested. A suspension of the drug in cod-liver oil gave promising results in the treatment of burns.

(*b*) *Immunity in monkey malaria*—Experiments with the cross circulation technique yielded the following results :—

(*i*) An immune monkey retains its immunity to *P. knowlesi* even when nearly 80% of its blood is replaced by that of a normal monkey.

(*ii*) A normal monkey acquires immunity when 75% of its blood is replaced by that of an immune monkey; if the replacement is 50%, immunity may or may not be acquired; if it is 33 % or less, immunity is not acquired.

(*iii*) If 70% of blood from a freshly infected monkey is replaced by immune blood, the animal passes into a stage of chronic infection.

The experiments show that both cellular and humoral agencies play a part in conferring immunity in malaria infections.

The proposals for 1942-43 are to continue and extend investigation on (*i*) the pharmacology of sulphanilamides with special reference to the treatment of wounds and burns, and (*ii*) the pharmacology of antimalarial drugs and the problem of malarial immunity.

Assam medical research society (malaria section).

An account of the activities of the Society was given by Dr. D. K. Viswanathan.

(*a*) *Malaria Surveys*—Surveys were carried out in two collieries, two tea estates and in Sadiya, the headquarters of the Sadiya Frontier Tract, and in two urban areas.

(*b*) Spraying-killing of adult mosquitoes was carried out in Limbuguri Tea Gardens and in Destipur, a rural centre. Estimations of the age of *A. minimus* caught in houses sprayed bi-weekly and once a week and also in an adjoining unsprayed garden, have been made and the results are being analysed. There was a marked reduction in the infection rate in *A. minimus* in the sprayed lines, and the fever rate remained at the same low proportion to which it has been reduced by this measure in the previous year.

(*c*) Studies of infant malaria parasite indices were continued. Analysis of 1,697 blood slides from infants show that:—

(*i*) There is a well-marked period of transmission from May to January.

(*ii*) The period of transmission of *P. vivax* is from May to July.

(*iii*) *P. falciparum* is the most prevalent species throughout the year, particularly in the last quarter.

(*iv*) There is some degree of inherited immunity in infants below two months.

(*v*) Infants at least four months' old show a tendency to relapses in April.

Since infants below two months show some evidence of immunity, those of two to three months of age furnish the best index of the quantum of seasonal transmission.

(*d*) *Vector species in Assam*—Although *A. minimus* is the most important vector throughout Assam, *A. annularis* and *A. maculatus* play some role in certain of the areas studied.

(*e*) *Entomological research*—Out of 5,120 *A. minimus* dissected during the twelve months, July 1940 to June 1941, 83 guts and 72 glands in 154 specimens were positive, giving a sporozoite rate of 1.4%, oocyst rate of 1.6%, and a total infection index of 3.0%.

Proposals for new enquiries.

(*a*) *Southern India Branch of the Malaria Institute of India*—The Director, Malaria Institute of India, proposed that the malaria research unit of the Rockefeller Foundation now working in Coonoor, where laboratory accommodation

has been provided by the Pasteur Institute of Southern India, be taken over with effect from 1st April, 1942, as the Southern Branch of the Malaria Institute of India. If this proposal is accepted, the Rockefeller Foundation are prepared (subject to confirmation by their New York office) to hand over to the Institute the entire equipment of the research unit, the mosquito colonies and the experimental animals. The Pasteur Institute Committee have unanimously and with appreciation agreed to afford to the Malaria Institute of India all the facilities granted to the Rockefeller Foundation, and the Government of Madras have welcomed the proposal. The advantages which would be gained by this arrangement can scarcely be exaggerated. Malaria conditions in Southern India are very different from those in the Punjab, and the advisability of establishing a Southern India Branch of the Institute has already been stressed by Sir Rickard Christophers and Colonel Sinton. The equipment valued at approximately Rs. 36,000 is in perfect condition and is unobtainable in India under present circumstances.

(*b*) *Study on the bionomics of A. minimus*—It is proposed to investigate whether transmission in the cold weather is of such an order as to require control, and whether lack of transmission in the cold weather in certain areas is due to the paucity and short span of adult prevalence under suitable conditions.

(*c*) *Malaria survey in four representative areas in Cachar District.* It is proposed to carry out detailed malaria surveys in four areas selected in different parts of Cachar District, Assam.

(*d*) *Enquiry into the adult habits of A. varuna*—This enquiry was sanctioned for the financial year 1940-41, but the work had to be closed down when Mr. Senior White proceeded on military service. On his return to his previous post, he proposes to continue the enquiry.

(*e*) *Research on filariasis in Orissa Province*—It is proposed to carry out a survey with the object of determining the incidence of filariasis and making recommendations for its control and treatment.

The Committee made the following recommendations :—

EXISTING ENQUIRIES.

(*a*) Malaria Institute of India (Research Section)—The Committee approved the proposals put forward.

(*b*) *Malaria transmission enquiry*—The Committee noted that this has now terminated.

(*c*) *Chilka Lake weeds enquiry*—The Committee noted that this enquiry is now terminated.

(*d*) *Protozoal parasites enquiry*—The Committee recommended the continuance of this enquiry in so far as it concerns the parasites of malaria.

(*e*) *Enquiry on a species of avian malaria*—The Committee did not recommend the continuance of this enquiry.

(*f*) *Filariasis enquiry under Dr. Sundar Rao*—The Committee recommended the continuance of the enquiry, but suggested that its principal activity should be the investigation of methods of treatment.

(*g*) *Filariasis enquiry under Dr. Menon and Dr. Ramamurti*—The Committee did not recommend the continuance of this enquiry.

(*h*) *Pharmacological enquiry*—The Committee recommended the continuance of this enquiry in so far as it concerned the investigation of antimalarial drugs.

(*i*) *Assam Medical Research Society*—The Committee expressed its appreciation of the work described in Dr. Viswanathan's report.

PROPOSED ENQUIRIES.

(*a*) *Southern India Branch of the Malaria Institute of India*—The Committee expressed its appreciation of the extremely generous offer of the Rockefeller Foundation to present the entire equipment of its research unit in Coonoor as a free gift to the I. R. F. A. It noted that since the budget of the Malaria Institute (Research Section) for 1942-43 had been reduced by Rs. 5,675, the increased expenditure involved in taking over the Rockefeller unit would be less than Rs. 5,500. The Committee were unanimously in favour of accepting the offer and recommended the proposal for favourable consideration.

(*b*) *Study on the bionomics of A. minimus*—The Committee recommended this proposal for favourable consideration, but considered that its adoption should be conditional on the provision of Rs. 20,000 by the Assam Government for the continuance of the Society in its present form.

(*c*) *Malaria survey in Assam*—The Committee did not recommend the adoption of this proposal.

(*d*) *Enquiry into the adult habits of A. varuna*—The Committee noted that Mr. Senior White expected to complete this investigation during 1942-43 and recommended the proposal for favourable consideration.

(*e*) *Research on filariasis in Orissa Province*—The Committee did not recommend the adoption of this proposal.

REPORT OF THE NUTRITION ADVISORY COMMITTEE.

The Committee met on the 15th December, 1941, and considered the following :—

1. THE PRODUCTION OF PULSES IN INDIA.

The Committee took note of reports on soya bean submitted by various members. These were discussed with reference to the recommendation contained in the 1937 Report of the Committee, which reads as follows:

"*Soya bean*—The nutritive value of soya bean has been studied by experiments on animals and also by controlled experiments on school children. The general conclusion is that soya bean, considered as a supplement to typical Indian diets, is not of outstanding value; it does not appear to have any advantage over various common pulses which have long formed part of the diet of the Indian people. While it would be advisable that the results obtained in the Coonoor Laboratories should be confirmed elsewhere, existing data suggest that at present the encouragment of the production and consumption of soya bean need not be made a prominent part of nutritional and agricultural policy in India".

While the validity of this recommendation was questioned by certain members of the Committee, it was agreed that there was insufficient unanimity among the members of the Committee to justify a further pronouncement on soya bean. It was decided that further work was necessary to elucidate the problem. Accordingly a sub-committee was appointed to suggest lines on which further experiments on soya bean might be carried out by different laboratories.

2. THE EDUCATION OF SCHOOL CHILDREN ABOUT FOOD AND NUTRITION.

A draft of Health Bulletin No. 30 "Food and Diet (written for children)" was considered and generally approved by the Committee. Various amendments and additions were suggested, including the following:—

More pictures and diagrams should be inserted, more reference should be made to cooking methods, the cost of different foods and the importance of regular meals. The diagram illustrating the difference between a good and a bad diet should be in terms of the intake for a child rather than of an adult.

The Nutrition Advisory Committee also pointed out the possibility of producing some even simpler propaganda material for adult educational classes should be considered.

3. DISTRIBUTION OF HEALTH BULLETINS NOS. 23 AND 28.

The following information was supplied to the Committee:—

"Health Bulletin No. 23 "The Nutritive value of Indian Foods and the Planning of satisfactory Diets" was first published in 1937, and 25,000 copies were printed. A second edition (revised and enlarged) was issued in 1938 and 15,000 copies were printed. These are now exhausted and a third edition (further revised and enlarged) of 15,000 copies has been printed.

Health Bulletin No. 28 on "Rice" was printed in June 1940. Of the 5,000 copies printed, 1,200 were issued free. The number of copies now available with the Central Publication Branch is 2,554".

4. NUTRITION WORK IN PROVINCES AND STATES.

The Committee took note of reports on nutrition work in Baroda, Bengal, Bihar, Burma, Hyderabad and the Punjab.

It considered the future development of nutrition work in Provinces and States and expressed the view that such work should be continued after preliminary surveys of diet and state of nutrition have been made. The first task of the nutrition officer is to carry out diet surveys and gauge the extent of malnutrition. His next task is to attempt to improve the diet of the population concerned, using the knowledge he has gained about its defects. There is obviously no point in making extensive surveys unless some use is to be made of the findings and this is possible only if the health department concerned continues to carry out nutrition work. The basic idea is that every health department should include a specialist on nutrition, just as it may include a specialist on leprosy, malaria or infant welfare, and it is advantageous that the nutrition specialist should have a unit under his charge established on a permanent basis. This is in line with practice in various other countries.

It is undesirable that the nutrition officer should be called a "nutrition survey officer" since this suggests that he is employed only for a specific piece of field work.

When a nutrition officer has received training and has gained experience of nutrition problems in his Province or State, he should if possible be retained in this branch of the Health Department. If he is removed to a different sphere of work, another worker must be trained and he must gain experience, so that loss of continuity results.

The Committee was of the opinion that "Nutrition Committees" should be established in the various Provinces. One such Committee already exists in Bengal.

5. Training course in nutrition in Coonoor.

The usual course was given in April-June, 1941, the class including 26 members. This was the fifth annual course.

6. The production of a substitute for cod liver oil.

Shark liver oil is now being produced in the Madras Presidency (Calicut), Bombay, Travancore and Baroda, total production amounting to considerable quantities. Rapid progress has been made in this newly created industry and it appears that the lack of cod liver oil resulting from cessation of imports can easily be made good, as far as vitamin A is concerned, by developing indigenous supplies of fish liver oil rich in vitamin A.

7. Existing and proposed enquiries.

The Committee recommended the continuation of the following enquiries :—

(*a*) Nutrition research under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, Coonoor, and additional nutrition enquiry.

(*b*) Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(*c*) Nutrition investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

It was suggested that the I. R. F. A. support of this enquiry should be limited to a further two years, after which it is hoped that the Bengal Government will make itself responsible for financing any further work on this subject.

(*d*) Studies on human nutrition with Indian diets under Dr. K. P. Basu at the Dacca University.

(*e*) Enquiry into calcium metabolism in health and disease, under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(*f*) Enquiry into malnutrition of Bengali boys with reference to vitamin A deficiency under Professor N. M. Basu at the Presidency College, Calcutta.

It was recommended that the enquiry should concern itself with laboratory rather than clinical investigations.

(*g*) Enquiry into malnutrition of Bengali boys with reference to vitamins B & C deficiency under Professor N. M. Basu at the Presidency College, Calcutta.

It was recommended that the enquiry should concern itself with laboratory rather than clinical investgiations.

(*h*) Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lieut.-Col. E. W. O. G'. Kirwan, I.M.S., Professor of Opthalmic Surgery, Medical College, Calcutta.

(*i*) Studies on the metabolism of vitamin A and carotene under Dr. G. Sankaran, at the All-India Institute of Hygiene and Public Health, Calcutta.

(*j*) Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay.

(*k*) Enquiry on the formation functions and variations of plasma proteins in health and disease under Dr. N. C. Datta at the Grant Medical College, Bombay. Bombay.

(*l*) Enquiry on the nutritive value of fish liver oils obtained from fish along the Bombay sea coast under Professor S. P. Niyogi at the Seth G. S. Medical College, Bombay.

It was recommended that this enquiry should continue next year provided that satisfactory liaison is established with the Department of Fisheries, Bombay, or the Zoological Survey of India in order to obtain information on the life history of different species of fish.

The following new proposals were recommended :—

(*a*) Enquiry into the preparation of vitamin B_1 concentrates under Dr. B. Ahmad, Professor in Charge, Department of Organic and Biochemistry, Punjab University, Lahore.

It was recommended that the work on yeast carried out under this enquiry should be co-ordinated with work proceeding in the Nutrition Research Laboratories, Coonoor.

(*b*) Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(*c*) Assessment of physical fitness of pre-school children under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta.

It was recommended that the enquiry should be initiated on a smaller scale than that proposed by Dr. L. S. Ghosh.

The following enquiries should receive favourable consideration if funds permit.

(*a*) Enquiry on the biological value of different pulses commonly used in India by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Patna.

(*b*) Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Dr. M. N. Rudra at the Prince of Wales Medical College, Patna.

REPORT OF THE PLAGUE ADVISORY COMMITTEE

The Committee met on 16th December, 1941.

PLAGUE ENQUIRY UNDER THE DIRECTOR, HAFFKINE INSTITUTE, BOMBAY.

(*a*) *Chemotherapy of plague*—The results of treatment of 267 cases of plague at Latur were considered and it was noted that a large reduction in case mortality was obtained by the use of sulphathiazole and sulphapyridine in comparison with mortality under ordinary treatment. With sulphathiazole case mortality was 22.4% and sulphapyridine 30%; a control series relating to previous experiments had a case mortality of 53.6%. The respective figures in septicaemic cases were 41.9%, 57.5% and 90.6%. Sulphathiazole appeared to give the best results. The opitmal dosage was ascertained.

(*b*) *Serological studies*—Serological studies of strains of different types was continued in relation to selection of strains for vaccine and serum production.

(*c*) *Studies on plague vaccine*—Investigations were carried out on agar and broth grown vaccines prepared from strains of different types and killed by different methods. Little difference was shown in vaccines killed by heating at 54°C, by merthiolate (1 in 5,00,000) and sulphathiazole (1%), alcohol killed vaccines showed little protective power.

The protective value of live avirulent vaccines, as estimated by the protective dose for mice, was higher than that of killed vaccines prepared from the protective avirulent strains or other strains.

(*d*) *Anti plague serum*—Anti-plague serum was prepared by strains selected in relation to their antigenic properties and will be tested in the field during the coming months.

(*e*) *Other observations* were made on new methods of preparing media for growth of Past. pestis and on the production of strains of varying degrees of virulence and protective power.

Discussions and recommendations.

The Committee discussed the work on chemotherapy of plague and recommended that this be continued with special reference to field trials of sulphathiazole and other sulphanamide derivatives. It was considered advisable that the value of combined treatment with serum and the chemotherapeutic agents should be tested.

A detailed discussion on the methods of preparation of anti-plague vaccine took place and it was recommended that special attention should be given to investigations on the following points:—

(1) The suitability of agar-grown killed vaccines prepared from strains of the protective avirulent type, either classical strains or laboratory produced strains, in which virulence has been reduced in varying degree.

(2) The relative toxicity of agar-grown vaccines made from strains of different types as compared with broth-grown vaccines with similar content of antigenic material and that suitable dosage for such vaccines should be determined on volunteers. The Committee was of opinion that vaccine of the live avirulent type was not suitable for use in India owing to practical difficulties in relation to its production and distribution.

The Committee recommended that the grant of Rs. 21,982 asked for should be sanctioned but that, in the event of the field trials of combined treatment with serum and sulphathiazole not showing any advantage over chemotherapy alone, during the remaining months of the present financial year, the manufacture of anti-plague serum be discontinued and the allotment for this, amounting to Rs. 2,634 be deducted.

Proposals for a new plague enquiry in Salem district under the Director of Public Health, Madras.

The proposals for this enquiry were discussed in detail and the Committee noted that they included hospital treatment of plague cases which was already being financed by the I. R. F. A. elsewhere, and work on fumigation on which suitable information had been obtained during the Cumbum Valley Enquiry towards which the Association had previously contributed.

It was recommended that in the event of the Madras Government providing funds for the enquiry a contribution should be made by the Association towards the cost of items (*c*), (*d*) and (*f*) of the proposals which refer to matters of general research interest, *viz.* recrudescence of infection, parasitology and study of sylvatic plague.

REPORT OF THE MATERNAL MORTALITY COMMITTEE.

The Committee held its meeting at 2 p.m. on December 15th, 1941 in New Delhi.

Dr. Napier pointed out to the Committee the necessity, in view of the present financial position of the I. R. F. A., for reviewing the proposals in the light of their immediate necessity and of their direct or indirect bearing on the war effort.

A. Existing enquiries.

1. The Committee considered Dr. Napier's report on his enquiry into the anaemias and strongly supported the continuance of this enquiry.

Dr. Napier also drew the attention of the Committee to the following publications:

(i) Indian Medical Research Memoir No. 33 on " Anaemia of Pregnancy in Calcutta ".

(ii) Indian Research Fund Association Memorandum on Anaemia of Pregnancy in India (in the press).

The Committee welcomed these two publications which they considered would prove invaluable to other research workers in this field.

2. The Committee noted that the enquiry into the anaemia of pregnancy in Kanara district under the Director of Public Health, Bombay, was being discontinued at the end of the year.

3. Dr. L. Ghosh amplified her report on the study of the blood picture in normal pregnancy at the All-India Institute of Hygiene and Public Health, Calcutta, which had been circulated to the Committee. It was considered that the enquiry would produce useful results. Its continuance during the next year was recommended. It was thought that this investigation would be completed within the next budget period.

4. The pregnancy and follow up survey in Calcutta under Dr. L. Ghosh was then discussed by the Committee. The number of infants about whom full information was available was small, but the enquiry had nevertheless produced some useful data. This enquiry will be complete within the present financial year.

5. Dr. Purandare gave an account of his enquiry into the toxaemias of pregnancy in Bombay, which is being carried out at his own expense. The Committee heard with satisfaction that the enquiry would be continued next year.

B. New enquiries.

1. A proposal for an enquiry into the anaemia of pregnancy and malnutrition in Lahore was submitted by Major Taylor. After discussion the Committee were of opinion that the collection of information of this kind in Northern India will be complementary to similar investigations carried out in other parts of the country. The Committee recommended that this enquiry should be undertaken provided there was a reasonable prospect of Major Taylor being able to remain in charge of it throughout the year, and that it was carried out in collaboration with the Haematological Unit at the Calcutta School of Tropical Medicine. A research worker who will be available for this work has already been trained in Calcutta.

2. Two enquiries on the bacteriology of puerperal morbidity, submitted by Dr. S. K. Chatterji, Patna, and Dr. J. Jhirad, Bombay, were considered together. The Committee noted that little or no work had been done on this subject in India and therefore considered that an investigation of this nature would be very valuable. They accepted in principle the enquiry submitted by Dr. Jhirad and recommended that it be favourably considered by the S. A. B. They thought it was essential before commencing this enquiry to ascertain (i) that Dr. Soman's services would be available throughout the year, (ii) that the necessary laboratory accommodation would be provided at the Haffkine Institute, and (iii) that well-trained laboratory attendants could be included in their staff. It was thought that Col. Sokhey's assurance of collaboration should be obtained.

The Committee did not recommend the enquiry submitted by Dr. S. K. Chatterji.

C. Completed enquiries.

Copies of the report on an investigation into the causes of maternal mortality in Bombay by Dr. Jhirad were distributed to the members of the Committee. The Committee were informed that the report on the investigation carried out under Dr. Neal Edwards in Delhi was under preparation and that it would shortly be ready for the press.

The question of publishing a popular bulletin embodying the findings of the three maternal mortality enquiries in Calcutta, Bombay and Delhi as recommended by this Committee in December 1939 was considered. It was agreed that this was not an appropriate time to issue a bulletin. They recommended that this matter should be brought up again for consideration at a subsequent meeting.

D. Infant morbidity and mortality sub-committee.

Last year, the S. A. B. on the recommendation of the Maternal Mortality Advisory Committee, appointed a sub-committee to prepare a memorandum on the conduct of enquiries into the causes of infant morbidity and mortality. A note prepared by this sub-committee was presented by Dr. Raja.

It was noted that the sub-committee considered the preparation of a memorandum unjustifiable until actual experience in carrying out an enquiry of this nature had been gained, and that before proposals for a detailed enquiry into the causes of infant morbidity and mortality could be formulated certain preliminary steps were necessary, (1) an attempt to improve vital statistics relating to infant mortality, (2) the utilisation of the information already existing in the health departments in the provinces.

The definitions of 'stillbrith' and 'neonatal death' given in the note prepared by the sub-committee were discussed and the Committee agreed that they should be recommended to the Public Health Commissioner for adoption by health authorities in India.

The Committee then considered three proposals for preliminary inquiries put forward by the sub-committee.

(1) An enquiry into the causes of infant mortality in Delhi under Dr. Jean M. Orkney, W.M.S.

This enquiry is expected to supplement the data collected under Dr. L. Ghosh's pregnancy follow up survey in Calcutta and to indicate how far the

resources of the public health departments can be utilised in carrying out a detailed enquiry. The Committee recommended this enquiry.

(2) An enquiry into the maturity range of infants under Dr. L. Ghosh, W.M.S., Calcutta.

An enquiry of this nature is also essential before a detailed investigation can be carried out, and the limited enquiry for this purpose under Dr. L. Ghosh in Calcutta was therefore recommended.

(3) A proposal for a rural pregnancy follow up survey in the Singur Health Unit under Dr. L. Ghosh, was next considered. While the Committee were of opinion that the information to be collected would be very useful, they felt that the routine recording of such facts should be within the range of activity of the public health authorities in the areas concerned. It was decided, however, to recommend the proposal for the consideration of the S. A. B. in view of the fact that the All-India Institute of Hygiene and Public Health, Calcutta, are unable to provide the necessary funds for such an investigation.

E. Import of dried milks for infant feeding.

The Committee was asked to consider two questions in connection with the need for importing into India dried milk for the feeding of infants.

(*a*) Should Government permit the import of dried milk from Dollar countries to supplement imports from Sterling countries? Government will only permit imports from Dollar countries if it is shown that the import is essential to the health of infants.

(*b*) If so, what fraction of pre-war imports from the United Kingdom should be allowed to be imported from Dollar countries?

The Committee considered that the importation of a certain amount of dried milk into India for infant feeding is essential, and that the policy should be to maintain imports of at least 50 per cent of 1939. It was agreed that no distinction between the various proprietary brands of dried milks was justifiable on medical grounds, and that as long as dried milk up to this quota is available from countries on the Sterling standard there is no necessity to import from countries on Dollar Standard.

In order to restrict the use of dried milks by individuals and institutions for purposes other than infant feeding the Committee recommend that the major part of the imported dried milk should be milk specifically prepared for infant feeding.

Much of the popularity of dried milks in infant feeding is due to lack of knowledge of how to prepare fresh milk mixtures, and in view of the danger of supplies of imported dried milk being cut off, the Committee recommended that a small sub-committee consisting of a pediatrician, a health worker, and a nutritionist with power to co-opt should be appointed, (*a*) to draw up pamphlet on the use of fresh milk mixture for infant feeding, and (*b*) to consider methods of making this information readily available to that section of the community by whom dried milks are generally used.

REPORT OF THE SUB-COMMITTEE ON SOYA BEAN APPOINTED BY THE NUTRITION ADVISORY COMMITTEE AT ITS MEETING HELD IN NEW DELHI ON THE 15TH DECEMBER, 1941.

The Sub-committee met on the 15th December, 1941.

Present:

Dr. K. P. Basu
,, B. C. Guha
,, V. N. Patwardhan
,, K. C. K. E. Raja
,, W. R. Aykroyd.

The committee was of the opinion that a coordinated investigation, to be carried out in a number of different laboratories, would help to solve differences of opinion. The project may be outlined as follows:

(1) Tests of the biological value of soya bean protein in different laboratories.

The sub-committee drew up a standardised method for carrying out tests by the metabolism and growth methods which all workers will follow. Details of the method will be circulated to the workers concerned by Dr. Aykroyd. The same sample of soya bean will be used for test in all the laboratories concerned, and the basal diets will be composed of similar materials. The results of the experiments will be analysed by the medical statistician.

(2) Growth tests with soya bean.

The principle of these experiments, as agreed on by the sub-committee, is as follows:—

Strictly comparable groups of young rats (minimum 12) are fed for 10-12 weeks on the following diets:

(*a*) a poor diet based on rice and resembling that consumed by the poorer classes, but containing no pulses.

(*b*) The same diet, but with 3 ozs. of rice replaced by an equivalent quantity of soya bean.

(*c*) The same as (*b*) using a number of other pulses instead of soya bean. The nature of these has been agreed on.

Experiments under (1) and (2) can be carried out in Bombay, Coonoor, Dacca by Drs. Patwardhan, Aykroyd and Basu respectively. If necessary, a fourth laboratory might be asked to cooperate. It was suggested that Dr. Ahmad in Lahore might undertake work in this field. Dr. Guha repeated that he *might* be able to undertake biological tests under (1) and (2).

Dr. Aykroyd would be responsible for the coordination of the scheme.

Human experiments.

The committee noted that these might be regarded as very difficult but nevertheless highly important.

(*a*) First, a simple attempt can be made to introduce soya bean in various forms into the diet of suitable institutions. This would not be a scientific

experiment, but would throw light on the question whether soya bean can easily be made acceptable and on suitable methods of preparation and cooking. It can be provided at the level of 2-3 ozs. daily and consumed in any convenient form—*e.g.* as an ordinary cooked pulse, or as flour made from roasted and unroasted beans which can be mixed with cereals, biscuits, etc., or added to other dishes and preparations. The giving of soya bean should be continued for 6-12 months. A rough idea of whether the soya bean supplement had any effect on health and development might be obtained, but the experiment would be a practical rather than a scientific one.

Drs. Aykroyd, Basu and Guha promised that they would attempt to find some suitable institution into which soya bean could be introduced. It would be necessary to obtain a supply of soya bean, which would be given free to the institutions in question.

(*b*) *Controlled experiments on school children*—These would be carried out roughly according to the following plan:

A suitable institution must be found and the children divided into three groups comparable as regards age distribution. It is presumed that the original diet of the institution is of the usual type—*i.e.* diet based on rice and some other cereal and containing 1-2 ozs. of pulses, vegetables of various kinds and little or no milk.

Group 1. Usual institutional diet.
,, 2. Same diet with the incorporation of 2-3 ozs. of soya bean in place of an equivalent quantity of cereal.
,, 3. do plus 2-3 ozs. of some other common pulse.

The sub-committee was not optimistic about the possibility of carrying a satisfactory controlled experiment on children. Dr. Aykroyd promised to make an attempt to arrange an experiment of this nature and Dr. Guha said he would look into the matter in Calcutta. The design of any experiment would be fully discussed with the medical statistician.

Expenses.

(*a*) *Laboratory work*—Drs. Basu, Guha and Patwardhan stated that they would require an assistant to carry out the work, who should be employed *for one year on Rs.* 150/- *per month.* Extra contingent expenditure would probably be met out of the funds of the laboratories or contingent grants available under I. R. F. A. enquiries. The Nutrition Research Laboratories would not require any extra grant.

(*b*) *Human experiments*—The only expenditure which may be immediately contemplated under this head is that necessary to purchase a stock of soya bean for supplying to institutions. About 500 lbs. could be obtained at a cost of approximately Rs. 50/-. The Nutrition Research Laboratories would not require an additional grant for carrying out any controlled experiment on school children which would be arranged.

Total expenses (annual).

Three assistants at Rs. 1,800×3	..	Rs. 5,400
Cost of soya bean	..	Rs. 50
	Total ..	Rs. 5,450

Addendum—The vitamin content of soya bean and other pulses will be carefully compared, using modern techniques. Particular attention will be given to the B group. Much of the work will be done in the Nutrition Research Laboratories and Dr. Basu in Dacca will also cooperate. No additional expenditure will be involved.

INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

During the year which has elapsed since the last meeting Volume XXIX of the *Journal* has been completed by the publication of the January, April, July and October (1941) numbers (4 numbers corresponding with the calendar year as decided last year).

A. (1) *Volume XXVIII (1940)—The Journal*—In accordance with the arrangement to make the Volume coincide with the calendar year, this Volume consisted of only 2 quarterly numbers, July and October, 1940, to allow of a new Volume commencing in January 1941. The average size was 317 pages of literary and tabular matter and 8 plates. This represents an increase of 24 pages on the amount of matter published quarterly and a decrease in the number of plates by 4 in comparison with the previous Volume. Both these numbers appeared in time.

(2) *The Memoirs*—No *Memoir* was published with this Volume.

(3) *The net cost of Volume XXVIII* was Rs. 6,234 (2 numbers only).

B. (1) *Volume XXIX (1941)—The Journal*—Four numbers to correspond with the calendar year have been published, commencing with the January 1941 number, the average size being 233 pages and 4 plates. All numbers appeared in time.

(2) *The Memoirs*—One *Memoir* (No. 33) on 'Anaemia in Pregnancy in Calcutta' by L. E. Napier and M. I. Neal Edwards is in the Press and will appear before the end of the calendar year (Nov.-Dec., 1941). A further *Memoir* (No. 34) on 'A Comparative Nutritional Survey of various Indian Communities' by D. C. Wilson and E. M. Widdowson is under consideration (February 1942?).

C. *Distribution*—

Indian subscribers	115	225		
Foreign subscribers	110			
Exchange list	178	229 less 50 suspended owing to war conditions		
Free list	121			
Voucher copies to advertisers, file, regn:, make-up, police, specimen copies to intending advertisers, propaganda, etc. ..	35 (approx.)=249			
TOTAL ..	559	or	509	
Balance left with Publishers for sale ..	141	or	191	
Total copies printed ..	700			

The Exchange list has been increased by 3 and the Free list by 1 over last year by addition of new addresses. There has been a rise of 4 in Indian and a fall of 26 in foreign subscribers, which according to the Publishers is due to war conditions as the majority of those cancelled are for enemy countries. Besides, about 50 foreign addresses on the distribution lists have also been suspended owing to the same cause. There has also been a decrease by 5 in voucher copies to advertisers. There has thus been a *total decrease of 73* in the distribution as compared with last year. A large number of publications are obtained in exchange for the I. J. M. R. for the I. R. F. A. Library, though some from enemy countries have been suspended.

D. *Distribution lists*—The distribution lists of the *Journal* were last revised in July 1938 and a new corrected alphabetical list showing exchanges has been printed and copies are available from the office of the Secretary for distribution to workers under the I. R. F. A.

E. *Number of copies printed*—From July 1940 it has been decided to print only 700 copies instead of 900, as this number is considered sufficient, according to former experience, to meet all deamands, and the saving estimated will be about Rs. 1,000 per year. Spare copies of Volumes I to VIII are available from the office of the Editor, Indian Journal of Medical Research, Kasauli.

F. *The cost of printing the Journal*—The following statement shows the cost of production during the last five years :—

Cost of printing the Journal only.

Number.	XXIII-XXIV 1936–37	XXIV-XXV 1937–38	XXV-XXVI 1938–39	XXVI-XXVII 1939–40	XXVII-XXVIII-XXIX 1940–41
April ..	3,369	4,468	2,415	3,276	5,137
July ..	4,459	4,907	4,744	4,212	3,932 } 700
October	3,931	4,622	3,801	4,611	4,717 } copies
January	5,141	4,572	3,652	3,640	3,571 } each.
Totals	16,900	18,369	14,612	15,739	17,357
Average pages	284	278	251	260	317

Recoveries.

April ..	1,881	1,534	2,965	1,628	1,452
July ..	1,381	1,534	429	1,628	1,451
October	1,381	1,185	1,048	838	963
January	1,466	1,185	1,048	838	963
Totals ..	6,109	5,438	5,490	4,932	4,829

G. *Net cost of the Journal and Memoirs*—The following statement shows the net cost of the production of the *Journal* and *Memoirs* during the last five years :—

			Journal.		
April	1,488	2,934	549	1,648	3,685
July	3,141	3,373	4,316	2,584	2,481
October	2,613	3,437	2,752	3,773	3,754
January	3,675	3,387	2,603	2,802	2,608
Totals	10,917	13,131	9,122	10,807	12,528
			Memoirs.		
		No. 29	No. 30	No. 31	
		2,369	2,849	1,754	
				No. 32	
				1,632	
Grand total	10,917	15,500	11,971	14,193	12,528

For convenience of accounting all receipts are credited to the *Journal* account and the figures given for the *Memoirs* are the actual cost of production.

The net cost during 1940-41 after deduction of receipts was Rs. 12,528. All Publishers' bills for 1940-41 were submitted in time to make payments within the financial year.

H. *Financial position for the year 1941-42*—The budget allotment of the year was Rs. 12,000 for the cost of 4 quarterly numbers of the *Journal* and Rs. 2,250 for any *Memoir* which might be published. Against this the actual and estimated expenditure is as follows :—

1.	Volume XXIX, No. 2, April 1941 ..	729 (actual)
2.	„ „ No. 3, July 1941	2,206 (actual)
3.	„ „ No. 4, October 1941 and Volume XXX, No. 1, January 1942, @ Rs. 3,000 each (700 copies)	6,000 (estimated)
4.	Memoir No. 33	1,865 (estimated)
5.	Memoir No. 34 (under consideration) ..	1,500 (estimated)
	Total ..	12,300 (estimated)

The saving anticipated would be Rs. 1,950.

J. *General*—Publication of the *Journal* has been continued on the lines laid down at the Conference of 1932 and the services of the members of the Editorial Board have been made full use of by referring to them all papers dealing with the subjects in regard to which they have been nominated.

The efforts made to keep the *Journal* at manageable size by limiting the size of papers to what might be considered reasonable in relation to their relative importance have resulted in a considerable reduction in size as compared with the previous year. Many contributors have co-operated in this and have made reasonable reductions in their papers when asked to do so.

In Volume XXIX (January 1941 to October 1941) out of 92 articles published 33 were on nutrition, 19 on bacteriology, 10 on biochemistry, 6 on haematology and 5 each on epidemic dropsy and entomology.

K. *Budget estimate for 1942-43*—The following is the estimated cost of publication of the *Journal* during the year 1942-43 allowing for 700 copies of the usual 4 numbers and 1 *Memoir* which may possibly be received :—

4 quarterly issues of the *Journal* @ Rs. 3,000	..	Rs. 12,000
1 *Memoir*	..	Rs. 2,250
Total	..	Rs. 14,250

As the effort to reduce the size of the *Journal* and *Memoirs* by limitation of length of papers has had some effect in reducing cost, some reduction, say Rs. 2,000, may be made in the above estimate.

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1941.

A. *Indian Journal of Medical Research and Indian Medical Research Memoirs.*

ACHARYA, B. N., NIYOGI, S. P., and PATWARDHAN, V. N.—Balanced Diets. Part III. The Effect of Parching on the Biological Value of the Proteins of some Cereals and Pulses (Vol. 30, No. 1).

AYKROYD, W. R., and KRISHNAN, B. G.—Rice Diets and Beriberi (Vol. 29, No. 3).

AYKROYD, W. R., and KRISHNAN, B. G.—Infantile Mortality in the Beriberi Area of the Madras Presidency (Vol. 29, No. 4).

BASU, N. M., and DE, N. K.—Assessment of Vitamin A Deficiency amongst Bengalees and Determination of the Minimal and Optimal Requirements of Vitamin A by a Simplified Method for Measuring Visual Adaptation in Dark (Vol. 29, No. 3).

BHAVE, P. D., and BOPAIYA, M. S.—Diet Surveys and Investigations of Haemoglobin Levels in Coorg (Vol. 30, No. 1).

CHOPRA, R. N., BOSE, B. C., and CHOPRA, I. C.—A Preliminary Study of the Pharmacological Action of the Glucoside of *Cerbera odollam* (Vol. 30, No. 1).

CHOPRA, R. N., and CHAKRAVARTI, M.—A Preliminary Note on the Pharmacological Action of the Alkaloids of *Rauwolfia serpentina* (Vol. 29, No. 4).

CHOPRA, R. N., CHOPRA, G. S., and CHOPRA, I. C.—*Cannabis sativa* in Relation to Mental Diseases and Crime in India (Vol. 30, No. 1).

CHOPRA, R. N., GUPTA, J. C., and CHOPRA, G. S.—Pharmocological Action of the Essential Oil of *Curcuma longa* (Vol. 29, No. 4).

CHOPRA, R. N., GUPTA. J. C., and CHPORA, I. C.—A Preliminary Note on the Pharmacological Action of the Glucosides from *Paris polyphylla* Sm. Vol. 30, No. 1).

DATTA, N. C.—Metallic Contamination of Foodstuffs. Part IV. Investigations on Contamination of Foodstuffs with Copper from Brass and Bronze Vessels and the Metabolic Experiments on the Absorption and Excretion of Copper in Rats (Vol. 29, No. 4).

DHARMENDRA—Complement-Fixation by Leprous Sera after Absorption by Various Acid-fast Bacilli (Vol. 29, No. 3).

DHARMENDRA—The Immunological Skin Tests in Leprosy. Part I. The Isolation of a Protein Antigen of *Mycobacterium leprae* (Vol. 30, No. 1).

DHARMENDRA and LOWE, J.—The Immunological Skin Tests in Leprosy. Part II. The Isolated Protein Antigen in Relation to the Classical Mitsuda Reaction and the Early Reaction of Lepromin (Vol. 30, No. 1).

DHARMENDRA and LOWE, J.—The Immunological Skin Tests in Leprosy. Part III. The Isolated Protein Antigen in Relation to the Antigens used by other Workers (Vol. 30, No. 1).

DOGRA, J. R.—Studies on Peptic Ulcer in South India. Part III. Experimental Production of Gastroduodenal Ulcer (Vol. 29, No. 2).

DOGRA, J. R.—Studies on Peptic Ulcer in South India. Part IV. Incidence of Peptic Ulcer in India with Particular Reference to South India (Vol. 29, No. 3).

GHOSH, B. N., DE, S. S., and CHAUDHURI, D. K.—Separation of the Neurotoxin from the Crude Cobra Venom and Study of the Action of a Number of Reducing Agents on it (Vol. 29, No. 2).

GHOSH, L. M., and MAPLESTONE, P. A.—An Improved Method of Growing Pure Cultures of Ringworm Fungi (Vol. 29, No. 4).

GIRI, K. V., and NAGANNA, B.—An Absorption Method for the Estimation of Nicotinic Acid Content of Animal Tissues and Blood (Vol. 29, No. 3).

GOKHALE, S. K.—Blood Urea Clearance in Normal Indians: A study based on the Examination of 110 Normal Indian Men (Vol. 29, No. 3).

GREVAL, S. D. S., and CHANDRA, S. N.—An Important Antigenic Difference between Haemagglutinogens M & N (Vol. 29, No. 3).

GREVAL, S. D. S., DAS B. C., and SEN GUPTA, P. C.—Preparation and Use of the Witebsky, Klingenstein and Kuhn (W. K. K.) Antigen (Vol. 29, No. 3).

KOCHHAR, B. D.—Nicotinic Acid in Blood and in Urine Vol. 29, No. 2).

KRISHNAN, K. V., and NARAYANAN, E. K.—Preparation of Peptone for Bacteriological Work (Vol. 29, No. 3).

LAHIRI, M. N.—On the Foetal Infection by *L. icterohaemorrhagiae* in a Rat (Vol. 29, No. 4).

LAL, R. B., DAS GUPTA, A. C., AGARWALA, S. P., and ADAK, B.—Investigatigations into the Epidemiology of Epidemic Dropsy. Part XIII. Application of the Biological Test to Modified Argemone Oil and its Derivatives (Vol. 29, No. 4).

LAL, R. B., DAS GUPTA, A. C., MUKHERJI, S. P., and ADAK, B.—Investigations into the Epidemiology of Epidemic Dropsy. Part XIV. Feeding Experiments on Human Subjects to test the Toxicity of some of the Derivatives and Modifications of Argemone Oil (Vol. 29, No. 4).

LAL, R. B., and DAS GUPTA, A. C.—Investigations into the Epidemiology of Epidemic Dropsy. Part XV. Incidence by Season (Vol. 30, No. 1).

LAL, R. B., RAJA, K. C. K. E., and SATYA SWAROOP—Statistical Enquiry into the Epidemiology of Cholera in Bengal. Part I. A General Review of the Epidemiological Features of Cholera in Different Parts of Bengal (Vol. 29, No. 3).

LAL, R. B., RAJA, K. C. K. E., SATYA SWAROOP, and BASAK, K. C.—Statistical Enquiry into the Epidemiology of Cholera in Bengal. Part II. Formation of Homogeneous Cholera Districts (Vol. 29, No. 3).

MENON, K. P.—*Staphylococci* in Vaccine Lymph (Vol. 29, No. 2).

MENON, T. BHASKARA, and RAMAMURTI, B.—The Behaviour of the Infective Larvae of *Wuchereria bancrofti* with Special Reference to their Mode of Escape and Penentration of Skin (Vol. 29, No. 2).

MUKHERJI, S. P., LAL, R. B., and MATHUR, K. B. L.—Investigations into the Epidemiology of Epidemic Dropsy. Part XII. Isolation of Active Substances from Toxic Oils (Vol. 29, No. 2).

NAPIER, L. EVERARD, and NEAL EDWARDS, M. I.—Anaemia in Pregnancy in Calcutta. (*Memoir* No 33—December 1941).

NAPIER, L. EVERARD, NEAL EDWARDS, M. I., and DAS GUPTA, C. R.—Haematological Studies in Indians. Part XIII. Normal Indian Women in Calcutta (Vol. 29, No. 2).

NAPIER, L. EVERARD, SEN GUPTA, P. C., and CHANDRA SEKAR, C.—Haematological Studies in Indians. Part XIV. The Measurement of the Red Cell Diameter: Standard Price-Jones' Curve for an Indian Population (Vol. 29, No. 4).

NIYOGI, S. P., PATWARDHAN, V. N., ACHARYA, B. N., and CHITRE, R. G.—Balanced Diets. Part II. Studies on the Nutritional Value of Fish (Vol. 29, No. 2).

NIYOGI, S. P., PATWARDHAN, V. N., and SIRSAT, M. V.—Studies on Basal Metabolism in Bombay. Part III. An Examination of the Factors Influencing the Basal Matabolism (Vol. 29, No. 2).

PATWARDHAN, V. N., and CHITRE, R. G.—Studies in Calcium and Phosphorus Metabolism. Part V. The alleged Influence of Vitamin D on the Absorption of Calcium from the Intestine: Experiments with Albino Rats (Vol. 30, No. 1).

RAJAGOPAL, K.—Dark-Adaptation Tests in Cases of Clinical Night-Blindness (Vol. 29, No. 2).

RAJAGOPAL, K.—Physical and Chemical Methods of Estimating Vitamin A in Shark and Saw-fish Liver Oils (Vol. 29, No. 3).

RANGANATHAN, S.—Calcium Intake and Flouorine Poisoning in Rats (Vol. 29, No. 4).

RANGANATHAN, S.—The Vitamin C Content of some Fish Oils (Vol. 29, No. 4).

RAO, M. V. RADHAKRISHNA, and RAO, M. NARASIMHA.—Haematological Investigations in South India. Part VI. The Red Cell Diameter in Healthy Europeans and Indians (Vol. 30, No. 1).

READ, W. D. B., and PANDIT, S. R.—Distribution of *V. cholerae* and El Tor Type Strains in certain Rural Areas in India (Vol. 29, No. 3).

ROY, A. C., and CHOPRA, R. N.—Lecithin and Haemolysis (Vol. 29, No. 4).

SATYA SWAROOP, RAJA, K. C. K. E., LAL, R. B. and BASAK, K. C.—Statistical Enquiry into the Epidemiology of Cholera in Bengal. Part III. Endemicity and Epidemicity of the Homogeneous Cholera Districts Vol. 29, No. 3).

SHOURIE, K. L.—Dental Caries in Indian Children (Vol. 29, No. 4).

SHOURIE, K. L., and SUNDARARAJAN, A. R.—The Effect of Milling on the Vitamin B_1 (Thiamine) and Nicotinic Acid Content of Indian Wheat Products (Vol. 30, No. 1).

SINHA, A. S.—Vasomotor Responses in Isolated Perfused Guinea-pig Lungs (Vol. 30, No. 1).

SMITH, R. O. A., HALDER, K. C., and AHMED, I.—Further Investigations on the Transmission of Kala-azar. Part IV. The Duration of Life and other Observations on 'Blocked' Flies (Vol. 29, No. 4).

SMITH, R. O. A., and AHMED, I.—Further Investigations on the Transmission of Kala-azar. Part V. An Enquiry into the Relation between Malaria and Kala-azar in a Rural Area (Vol. 29, No. 4).

SMITH, R. O. A., and AHMED, I.—Further Investigations on the Transmission of Kala-azar. Part VI. A Second Series of Transmissions of *L. donovani* by *P. argentipes* (Vol. 29, No. 4)

STRICKLAND, C., and ROY, D. N.—Myiasis-producing Diptera in Man (Vol. 29, No. 4).

SUNDARARAJAN, A. R.—The Vitamin B_1 Content of Human Milk (Vol. 29, No. 3).

SWAMINATHAN, M.—Further Studies on the Cyanogen Bromide Method of Estimating Nicotinic Acid in Biological Materials (Vol. 29, No. 2).

SWAMINATHAN, M.—A Flourimetric Method for the Estimation of Riboflavin in Foodstuffs (Vol. 30, No. 1).

SWAMINATHAN, M.—Fluorimetric Estimation of Riboflavin in Urine (Vol. 30, No. 1).

SWAMINATHAN, M.—Fluorimetric Assay of Riboflavin in the Urine and Tissues of Rats (Vol. 30, No. 1).

SWAMINATHAN, M.—Urinary Excretion of Vitamin B_6 by Rats (Vol. 29, No. 3).

SWAMINATHAN, M.—A Method for the Estimation of Vitamin B_6 in Urine (Vol. 29, No. 3).

TELANG, D. M., and BHAGWAT, G. A.—Studies in the Vital Capacity of Bombay Medical Students. Part I. Statistical Correlation with Physical Measurements (Vol. 29, No. 4).

VEERARAGHAVAN, N.—Elimination of Excess Nerve Tissue from Antirabic Vaccine (Vol. 29, No. 2).

VENKATRAMAN, K. V., KRISHNASWAMI, A. K., and RAMAKRISHNAN, C. S.—Occurrence of Vibrio El Tor in Natural Sources of Water in the Absence of Cholera (Vol. 29, No. 3).

VENKATRAMAN, K. V., and RAMAKRISHNAN, C. S.—A Preserving Medium for the Transmission of Specimens for the Isolation of *Vibrio cholerae* (Vol. 29, No. 4).

B. *Journal of the Malaria Institute of India.*

COVELL, G.—Antimalaria Operations in Delhi. Part III. (Vol. IV, No. 1).

DIKSHIT, B. B.—Malarial immunity in the Rhesus monkey. (Vol. IV, No. 2).

SENIOR WHITE, R.—Observations on the adult habits of *Anopheles fluviatilis* and *Anopheles veruna*. (Vol. IV, No. 1).

C. *List of papers published by workers under the I. R. F. A. in Journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

AYKROYD, W. R.—Indian Diets and Their Imrpovement. (Nutrition Abstracts and Reviews, in press).

AYKROYD, W. R.—Economic Aspects of the Problem of Nutrition in India. (The Indian Journal of Social Work, in press).

AYKROYD, W. R., and KRISHNAN, B. G.—Infantile Mortality and Beri-beri. (Current Science, 1941, 10, 3, p. 169).

AYKROYD, W. R., and VERMA. O. P.—Superficial Keratitis due to Riboflavin Deficiency. (I. M. G. in press).

BANERJEE, D. N.—Pulmonary oedema in Cholera (Jl. Ind. Med. Assoc. Vol. 11, 1941, p. 45).

BANERJEE, D. N.—Concentrated saline in the treatment of cholera. (A priliminary report). (Abstract published in the proceedings of the 29th

Indian Science Congress, Section of Medical and Veterinary Research, Baroda, 1942).

BANERJEE, S., and GUHA, B. C.—Urinary excretion of combined ascorbic acid in pulmonary tuberculosis. (Ann. Biochem. Expt. Med., Vol. I No. I.)

BASU, N. M. and DE, N. K.—Assessment of vitamin A deficiency etc. (Proc. Ind. Science Congress 1941).

BASU, N. M., and DE, N. K.—Storage of vitamin A in liver under different conditions etc. (Science and Culture (1941), VI, p. 672.)

BASU, N. M., and RAY, G. K.—Assessment of vitamin B_1, deficiency of persons living on diets of Bengalis. (Proc. 28th Indian Sc. Congress 1941).

BASU, N. M., and RAY, G. K.—Synaergism between vitamins B_1 and C. (Proc. 28th Indian. Sc. Congress 1941).

BASU, K. P., BASAK, M. N., and HALDER, M. K.—On the significance of S/N ratio in human urine and on the distribution of urinary sulphur on non-protein diet and on diets containing different amounts of proteins from rice and whole wheat. (Annals of Biochemistry and Experimental Medicine, Vol. I, No. 1).

BOPAIYA, M. S., and RAO, M. V. RADHAKRISHNA.—Note on "Tropical Ulcer" in Coorg. (I. M. G. in press).

CHOPRA, R. N., BADHWAR, R. L., and NAYAR, S. L.—Insecticidal and piscicidal plants of India. (J. Bombay Nat. Hist. Soc., June, 1941).

CHOPRA, R. N., and CHAKRAVARTY—Gastric acidity in chronic ulcerative colitis. (I. M. G., Vol. LXXVI, No. 10).

CHOPRA, R. N., CHOPRA, G. S., and CHOPRA, I. C.—A study of normal blood pressure in Indians. (I. M. G., Vol. LXXVI, No. 9).

CHOPRA, R. N., CHOPRA, G. S., and CHOPRA, I. C.—Minor drug habits of India, Parts I and II. (I. M. G. Vol. LXXVI, No. 10).

CHOPRA, R. N., CHOPRA, G. S., and CHOPRA, I. C.—Eucodal habit in India. (I. M. G. Vol. LXXVI, No. 11).

CHOPRA, R. N., and CHOPRA, I. C.—Treatment of chronic intestinal amœbiasis. (I. M. G. Vol. LXXVI, No. 11).

CHOPRA, R. N., CHOWHAN, J. S., and CHOPRA, I. C.—Sterilisation of snake venom preparations. (I. M. G., Vol. LXXVI, No. 10).

CHOPRA, R. N., DEMONTE, A. J. H., CHATTERJEE, B. C., and GUPTA, S. K.—Sulphanilylguanidine in cholera. (I. M. G., Vol. LXXVI, No. 10).

CHOPRA, R. N., GUPTA, J. C., BOSE, B. C., and GANGULY, S. K.—A preliminary study of the Rideal-Walker coefficient values of certain indigenous essential oils. (I. M. G., Vol. LXXVI, No. 10).

COCHRANE, R. G., MENON, K. P. and PANDIT, C. G.—"Further report on inoculation of monkeys with human leprosy material after splenectomy." (International Journal of Leprosy, October 1941.) (in press).

COCHRANE, R. G., RAJAGOPALAN, G., SANTRA, I., and M. PAUL RAJ.—A study of the Lepromin Reaction in children with special reference to contact. (Leprosy in India, Jan. 1941).

COVELL, G.—Social aspects of malaria. (Science and Culture, Vol. VI).

DAS GUPTA, B. M., and SIDDONS, L. B.—On a *Plasmodium* sp. the Malay Chestnut-bellied Munia (*Munia atricapilla atricapilla* Vieill) (*I. M. G.* Vol. LXXVI, No. 3.)

DAS GUPTA, B. M., and SIDDONS, L. B.—On a trypanosome of the White-throated Munia—*Uroloncha malabarica* (Linn.) (*I. M. G.*, Vol. LXXVI, No. 3.)

DAS GUPTA, G. C. and GUHA, B. C.—The effect of vitamin C and certain other substances on the growth of micro-organisms. (Ann. Biochem. Expt. Med., Vol. I, No. 1).

DE, S. S.—Crystalline haemolysine from cobra (Naja Naja) venom. (Science and Culture, Vol. VI, 1941, p. 675).

DEY, N. C.—Notes on common skin diseases III Ringworm of the scalp : Favus. (I. M. G. Vol. LXXVI, No. 7, July, 1941).

DEY, N. C., and MAPLESTONE, P. A.—Trichophyton crateriforme in India. (I. M. G. Vol. LXXVI, No. 7, July, 1941).

DHARMENDRA—" Tuvaraka " of the ancient Hindu Medicine is hydnocarpys wightiana and not Gynocardia Odorata or Taraktogenos kurzi. (Lep. in Ind. Vol. XIII, No. 2).

DHARMENDRA—Studies of the lepromin test (3) Preparation and standardisation of lepromin. (Lep. in Ind. Vol. XIII, No. 3).

DHARMENDRA—Studies of the lepromin test. (5) The active principles of lepromin is a protein antigen of the bacillus (Lep, in Ind. Vol XIII, No. 3).

DHARMENDRA, and JAIKARIA S.S.—Studies of the lepromin test. (2) Results of the test in healthy persons in endemic and non-endemic areas. (Lep. in Ind. Vol. XIII, No. 2).

GHOSH, B.—The effect of administration of toxic substances on ascorbic acid metabolism. (Ann. Biochem. Expt. Med., Vol. I., No. 1.)

GHOSH, B. N., DE, S. S., and CHAUDHURI, D. K.—Enzymes in snake venom. (Ann. Biochem. Expt. Med. Vol. I, 1941, No. 1).

GREVAL, S. D. S., CHANDRA, S. N., and CHATTERJI, D. N.—Taking Blood for Transfusion : Further Improvisations. (I. M. G., Vol. LXXVI, No. 7, July 1941).

GUHA THAKURTA, S. R.—Tuberculin Test—its value in the early diagnosis of Pulmonary Tuberculos. (I. M. G. Vol. LXXVI, No. 7, July, 1941).

LAHIRI, M. N.—Studies on *Leptospira icterohaemorrhagiae* in Rats in Bombay City (I. M. G. Vol. LXXVI, No. 9).

LAHIRI, M. N.—A note on the occurrence of Leptospirosis in Bombay. (I. M. G. Vol. LXXVI, No. 11).

LOWE, J. and CHATTERJI, S. N.—The onset of leprosy of the lepromatous type. (Lep. in Ind. Vol. XIII, No. 1).

LOWE, J., and CHATTERJI, S. N.—An acute onset of leprosy of lepromatous type in a man aged seventy. (Lep. in Ind. Vol. XIII, No. 1).

LOWE, J. and CHATTERJI, S. N.—An abortive case of leprosy (Lep. in Ind. Vol. XIII, No. 1).

LOWE, J, and CHATTERJI, S. N.—The development of leprosy in a child. (Lep. in Ind. Vol. XIII, No. 2).

LOWE, J. and DHARMENDRA—Studies of the lepromin test. (4) The early reaction to lepromin its nature and its relation to the classical Mitsuda reaction (Lep. in Ind. Vol. XIII, No. 3).

LOWE, J., DHARMENDRA, and SEN, N. R.—Epidemiological and clinical studies of leprosy in the Bankura district of Bengal (Lep. in Ind. Vol. XIII, No. 4).

NAPIER, L. E., and DAS GUPTA, C. R.—Haematological technique. (*I. M. G.* Vol. LXXVI, Nos. 1-4, 7, and 11-12).

NAPIER, L. E., and DAS PUTA, C. R.—A case of thrombocytopenic perpura (*I. M. G.* Vol. LXXVI, No. 5).

NAPIER, L. E., DAS GUPTA, C. R., and MAJUMDAR, D. N.—The treatment of Hookworm anaemia. (*I. M. G.* Vol. LXXVI, No. 1).

NAPIER, L. E. and SEN GUPTA, P. C.—Haematological technique. (*I. M. G.* Vol. LXXVI, No. 5).

PANJA, D.—Treatment of Tuberculosis verrucosa cutis by local application of creosote and salicylic acid. (I. M. G. Vol. LXXVI, No. 2, Feb., 1941).

PANJA, D.—Notes on common skin diseases IV Leucoderma. (I. M. G. Vol. LXXVI, No. 9, Sept., 1941).

PANSE, and PARANJPE—A preliminary note on an alkaloida[1] substance isolated from Carica Papaya seeds. (Rasayanam, 1941, Vol. I. No. 7)

PARIJA, P., and PARIJA; B.—Study of the weeds of the Chilka lake—I. (Proceedings of the Indian Science Congress abstract p. 166-167, 1941).

PARIJA, P., and PARIJA, B.—Algal succession on a rocky island, named Charaiguha in the Chilka Lake (Proceedings of the Indian Science Congress Abstract, 1942).

ROY, D. N., GHOSH S. M., and CHOPRA, R. N.—A comparative efficacy of different culicifuges under laboratory conditions. (Parasitology, Vol. 33, Sept. 1941).

ROY, D. N., GHOSH, S. M., and CHOPRA, R. N.—The mode of action of pyrethrum on cockroach. (J. of Exp. Biol., Vol. XVIII, Nov. 1941).

SAHA, K. C.—Nicotinic acid content of fish (Ann. Biochem. Expt. Med., Vol. I, No. 1).

SAHA, K. C.—Copper content of fish. (Ann. Biochem. Expt. Med., Vol. I, No. 1).

SAHA, K. C.—Available Iron in fish—Part II. The nature of iron in fish tissue. (Ann. Biochem. Expt. Med., Vol. I, No. 2).

SAHA, K. C., and GHOSH, N. C.—Nutritional Investigation on fish. (Ann. Biochem. Expt. Med., Vol. I, No. 2).

SAHA, K. C., MAZUMDAR, A. C., and GUHA, B. C.—Distribution of free and bound ascorbic acid and haemoglobin in blood of normal vertebrates. (Ann. Biochem. Expt. Med., Vol. I., No. 2).

SAHA, K. C., MAZUMDER, A. C., and GUHA, B. C.—Relation between blood ascorbic acid, ascorbigen and haemoglobin content at different stages of scurvy in experimental guinea-pigs. (Ann. Biochem. Expt. Med., Vol. I., No. 2).

SEN, P. K.—The treatment of Cavitation in tuberculosis of the lungs. (Trans. Med. Col. Re-Union, 1940).

SEN, P. K.—Is good health a protection against tuberculosis? (Cal. Mun. Gaz. Health Number, April, 1941).

SEN, P. K.—Early Diagonsis of Pulmonary Tuberculosis, (Trans. Med. Coll. Re-Union, 1941) (Under publication).

SEN GUPTA, A. B., SARKER, B. B., and GUHA, B. C.—A comparative study of the chemical and biological methods of estimation of vitamin C of cabbage juice. (Ann. Biochem. Expt. Med., Vol. I., No. 2.)

SLOAN, T. B. M., and ROY EBENEZER—Skin Grafts in Leprosy (Leprosy in India, October, 1941).

UKIL, A. C.—The interpretation of Lung radiograms in pulmonary tuberculosis. (Trans. Tub. Workers' Conference, 1940 and Trans. Cal. Med. Coll. Re-Union, 1940.

UKIL, A. C.—Some aspects of Public Health in India, Presidential Address, Section of Medical and Veterinary Research, 28th Ind. Sc. Cong., Benares, 1941.

UKIL, A. C.—Tuberculosis and the Nursing Service, (Journ. Beng-Tub. Associn. Vol. 6, No. 5, May, 1941.)

UKIL, A. C.—Early diagnosis and treatment of tuberculous enteritis, (Trans. Med. Col. Re-Union, 1941) (under publication).

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 16th and 17th December, 1941.

	Enquiries.	Budget allotments. Rs.
1.	Enquiry into the treatment of cholera (*a*) by transfusion of blood plasma, serum, etc. and (*b*) by sulphanilamide derivatives or other new compounds under the Director, School of Tropical Medicine, Calcutta	4,000
2.	Malaria Institute of India (Research Section)	29,461
3.	Nutrition Research Laboratories, I. R. F. A., Coonoor ..	84,887
4.	Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	5,424
5.	Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta ..	3,058
6.	Enquiry entitled "Studies on human nutrition with Indian diets" under Dr. K. P. Basu at Dacca University	4,992
7.	Enquiry on calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	2,833
8.	Enquiry into malnutrition of Bengali boys with reference to vitamins B and C deficiency under Professor N. M. Basu at the Presidency College, Calcutta	2,746
9.	Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lieut.-Colonel E. W. O'G. Kirwan, I.M.S., Professor Ophthalmic Surgery, Medical College, Calcutta	2,692
10.	Enquiry on the nutritive value of fish liver oils obtained from fish along the Bombay sea coast under Professor S. P. Niyogi at the Seth G. S. Medical College, Bombay	3,820
11.	Enquiry entitled "Studies on the metabolism of vitamin A and carotene" under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta	2,619
12.	Enquiry entitled "Studies in tests of physical fitness in Indians" under Dr. D. M. Telang at the Grant Medical College, Bombay ..	1,748
13.	Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	1,949
14.	Additional nutrition enquiry under the Assistant Director, Nutrition Research Laboratories, Coonoor. (*The cost to be met from the balance of Rs.* 30,000/- *annual grant sanctioned by the Governing Body over a period of five years, out of the special ten lakhs grant made by Government for antimalaria and nutrition work*)	21,951
15.	Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta	6,340

	Enquiries.	Budget allotments. Rs.
16.	Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras	2,190
17.	Plague researches under the Director, Haffkine Institute, Bombay	21,982
18.	Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta	10,000
19.	Enquiry into the incidence of positive Mantoux reactions and latent tuberculosis as judged by skiagrams in medical students and nurses under Major G. F. Taylor, I.M.S. at the K. E. Medical College, Lahore	500
20.	Epidemiological enquiry on tuberculosis at Saidapet under Dr. R. G. Cochrane, Chief Medical Officer, Lady Willingdon Leprosy Sanatorium, Chingleput, Madras	4,000
21.	Indigenous drugs enquiry under Dr. Khem Singh Grewal at the K. E. Medical College, Lahore	2,208
22.	Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay	2,502
23.	Enquiry into anaemia of women in Assam under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta	4,492
24.	Enquiry entitled "Study of the blood picture in normal pregnancy" under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta	3,025
25.	Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta	3,098
26.	Enquiry into medical mycology under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta	5,580
27.	Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta	4,686
28.	Enquiry into filariasis under Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Vizagapatam	2,022
29.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	1,505
30.	Kala-azar enquiry under the Director, Pasteur Institute, Shillong	18,544
31.	Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay	4,236
32.	Enquiry on the algae present in water reservoirs and their effect on purification methods and on public health under the Director, King Institute, Guindy, Madras	1,230
33.	Enquiry into methods for the manufacture of blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta ..	6,600
34.	Enquiry into the pathology and pathogenesis of splenomegaly under Dr. D. Govinda Reddy at the Medical College, Madras ..	2,727
35.	Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay	3,621

	Enquiries.	Budget allotments. Rs.
36.	Enquiry entitled "Haematological survey of poor class families in and around Lucknow" under Dr. V. S. Mangalik at the King George Medical College, Lucknow	1,185
37.	Enquiry entitled "preliminary studies on the virus of influenza" under the Director, King Institute, Guindy, Madras ..	2,138
38.	Enquiry entitled "Study of bionomics of *A. minimus* both directly and indirectly from the human response" under the Assam Medical Research Society.	4,236
39.	Enquiry into the adult habits of *A. varuna* under Mr. R. Senior White, Malariologist, B. N. Railway, Calcutta	354
40.	Southern India Branch of the Malaria Institute of India (Research Section)	11,132
41.	Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay	3,514
42.	Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna	1,650
43.	Plague enquiry in the Salem District under the Director of Public Health, Madras	2,500
44.	Indigenous drugs enquiry at the Biochemical Standardisation Laboratory, Calcutta	7,738
45.	Indigenous drugs enquiry under Bt.-Col. Sir Ram Nath Ghopra at the Drug Research Laboratory, Kashmir	3,372
46.	Enquiry into the occurrence of ascites in Hyderabad under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad-Deccan	2,178
47.	Enquiry on the action of snake venoms and their constitutents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	2,800
48.	Enquiry into the maturity range of infants under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health Calcutta	505
49.	Enquiry to determine the endemic foci of cholera in certain parts of Bengal and Madras Presidencies under Dr. K. C. K. E. Raja, Deputy Public Health Commissioner with the Government of India	2,628
50.	Enquiry into the isolation of atropa from *dhatura* under Dr. J. N. Ray at the Delhi University Laboratories.	2,900
51.	Enquiries into (1) tests of the biological value of soya bean protein, (2) growth tests with soya bean and (3) human experiments with soya bean to be carried out by Dr. Aykroyd, Dr. K. P. Basu, Dr. B. C. Guha and Dr. V. N. Patwardhan ..	5,450
52.	Enquiry into the causes of infant morbidity and mortality in Delhi under Dr. Jean M. Orkney; Director, Maternity and Child welfare Bureau, Indian Red Cross Society, New Delhi ..	1,100

	Grants.		Budget allotments. Rs.
(*i*)	Administration charges		35,766
(*ii*)	Contribution towards the cost of two professorships at the School of Tropical Medicine, Calcutta		26,400
(*iii*)	Contribution to the Imperial Institute of Entomology, London (£ 100)		1,350
(*iv*)	Contribution to the Bureau of Hygiene and Tropical Disesaes, London. (£ 150)		2,025
(*v*)	Contribution towards the cost of the Statistical Bureau under the Public Health Commissioner with the Government of India		6,650
(*vi*)	Five research fellowships of the value of Rs. 150/- p.m. each ..		9,000
(*vii*)	Indian Journal of Medical Research and Memoirs.		
	(*a*) Publication charges of the Journal ..	12,000	
	(*b*) Publication charges of the Memoirs ..	2,250	
	(*c*) Editor's office	4,388	18,588
(*viii*)	I. R. F. A. Library at the Central Research Institute, Kasauli ..		2,140
(*ix*)	Pay, including special pay, overseas pay and allowances and contributions towards passage, leave and pension or contributory provident fund of the officers of the Medical Research Department under the Association		1,50,000
(*x*)	Travelling allowance and certain contingent expenses for Medical Research Department officers employed under the I. R. F. A. and attached to central or provincial laboratories ..		2,000
(*xi*)	Pay and travelling allowance on transfer of the three workers in the Junior Research cadre		19,140
(*xii*)	Pay of two clerks at the School of Tropical Medicine, Calcutta ..		3,030
(*xiii*)	Clerical assistance to the Director, All-India Institute of Hygiene and Public Health, Calcutta		1,197
(*xiv*)	Travelling allowance of subordinate I. R. F. A. workers on transfer from one post to another		1,500
(*xv*)	I. R. F. A. contribution and interest towards the Contributory Provident Fund		14,000
(*xvi*)	Miscellaneous unforseen expenditure		8,000
(*xvii*)	Provision for imprest advance for enquiries		8,450

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1942.

Issued under the authority of the Governing Body,
Indian Research Fund Association,
New Delhi,
India.

Price per copy One Rupee.

Obtainable from the Secretary, Governing Body, Indian
Research Fund Association, Secretariat,
NEW DELHI (India).

With the compliments of

Lieut.-Colonel E. Cotter, C.I.E., I.M.S., Secretary, Governing Body and Scientific Advisory Board, Indian Research Fund Association.

NEW DELHI
INDIA.

CONTENTS.

	Page.
I. Composition of the Board	i
II. Composition of Advisory Committees	ii
III. Meetings held during the year	iii
IV. Researches carried out during the year	1—107

CHOLERA.

Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta	1—4
Enquiry to determine the endemic foci of cholera in certain parts of Bengal and Madras Presidencies under Dr. K. C. K. E. Raja, Offg. Deputy Public Health Commissioner with the Government of India	4—8

MALARIA.

Malaria Institute of India (Research Section), Kasauli/Delhi ..	9—11
Enquiry into the adult habits of *A. varuna* under Major R. Senior White, Malariologist, B. N. Railway, Calcutta	11—12

NUTRITION.

Nutrition researches under Dr. W. R. Aykroyd at the I. R. F. A. Nutrition Research Laboratories, Coonoor	13—18
Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	18—20
Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta ..	20—21
Studies on human nutrition with Indian diets under Dr. K. P. Basu at the Dacca University, Dacca	21—23
Enquiry into calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	23
Enquiry into malnutrition of Bengali boys with reference to vitamins B and C deficiency under Professor N. M. Basu at the Presidency College, Calcutta	24—27
Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lieut.-Colonel E. W. O'G. Kirwan, Professor of Ophthalmic Surgery, Medical College, Calcutta ..	27—28
Studies on the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta	28—29
Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay	29—33

Page

Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay .. .. .. 34—38

Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay .. .. .. 38—39

Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna .. .. 39—40

Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean to be carried out under Dr. K. P. Basu at the Dacca University, Dacca 41

Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay 42

Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. Bashir Ahmad, Professor of Organic and Biochemistry, Punjab University, Lahore .. .. 42—43

LEPROSY

Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta .. .. 44—46

Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras .. .. 46—53

PLAGUE.

Plague Research under the Director, Haffkine Institute, Bombay .. 54—59

TUBERCULOSIS

Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta .. .. 60—67

PHARMACOLOGY.

Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore .. .. .. 68—69

Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay .. .. .. 69—70

Indigenous drugs enquiry under Dr. J. C. Gupta, Professor of Pharmacology, School of Tropical Medicine, Calcutta .. .. 70—73

Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S. (R) at the Drug Research Laboratory, Jammu, Kashmir 73

Page

Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay .. .. 74—75

MATERNAL MORTALITY.

Haematological unit under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta .. .. 76—78

Study of the blood picture in normal pregnancy under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta .. .. 78—79

Enquiry into the maturity range of infants under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcusta 79

OTHER RESEARCHES.

Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta .. .. 80—81

Enquiry into medical mycology under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta .. .. 81—83

Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta .. .. 84—86

Enquiry into filariasis under Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Madras .. .. .. 86—87

Enquiries on filterable viruses and influenza virus under the Director, King Institute, Guindy, Madras .. .. 87—88

Kala-azar enquiry under the Director, Pasteur Institute, Shillong .. 88—91

Enquiry on the algae present in water reservoirs and their effect on purification methods and on public health under the Director, King Institute, Guindy, Madras .. .. .. 91—93

Enquiry into the pathology and pathogenesis of splenomegaly under Dr. D. Govinda Reddy at the Medical College, Madras .. 93—94

Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay .. .. 94—96

Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta .. 96—98

Enquiry into the occurrence of ascites in Hyderabad under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan .. .. 98—102

Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta .. .. 102—103

Enquiry into the isolation of atropa from *dhatura* under Dr. J. N. Ray at the Delhi University Laboratories, Delhi .. .. 103—104

Construction of a Pilot Plant for drying human plasma or serum at the All-India Institute of Hygiene and Public Health, Calcutta 104—105

Page.

Leptospirosis enquiry under Dr. M. N. Lahiri at the Haffkine Institute, Bombay .. .. 105—107

V. Reports of Advisory Committees on

Malaria .. 108—112

Nutrition .. 113—117

Plague .. 117—119

VI. Indian Journal of Medical Research and Indian Medical Research Memoirs .. .. 120—123

VII. Miscellaneous papers issued under the auspices of the Indian Research Fund Association .. .. 124—128

VIII. Programme of researches and miscellaneous grants recommended for the financial year 1943-44 .. .. .. 129—132

The Scientific Advisory Board consisted of the following :—

President :

Lieut.-General Sir GORDON JOLLY, K.C.I.E., K.H.S., I.M.S., Director-General, Indian Medical Service.

Members :

Lieut.-Colonel E. COTTER, I.M.S., Public Health Commissioner with the Government of India. (1st January to 26th July and 27th August to 31st December, 1942). *Secretary.*

Major C. A. BOZMAN, I.M.S., Offg. Public Health Commissioner with the Government of India. (27th July to 26th August, 1942). *Secretary.*

Major-General Sir JOHN TAYLOR, C.I.E., D.S.O., I.M.S. (Retd.), Director, Central Research Institute, Kasauli.

Dr. L. E. NAPIER, C.I.E., F.R.C.P., Director, School of Tropical Medicine, Calcutta.

Lieut.-Colonel S. S. SOKHEY, I.M.S., Director, Haffkine Institute, Parel, Bombay.

Major K. P. Menon, Offg. Director, King Institute, Guindy, Madras. (1st January to 14th June, 1942.)

Dr. C. G. PANDIT, Director, King Institute, Guindy, Madras. (15th June to 31st December, 1942).

Brigadier G. COVELL, C.I.E., I.M.S., Director, Malaria Institute of India, Kasauli.

Bt.-Colonel Sir RAM NATH CHOPRA, C.I.E., I.M.S. (Retd.), Director, Drug Research Laboratory, Jammu, Kashmir.

Dr. V. R. KHANOLKAR, Director of Laboratories, Tata Memorial Hospital, Parel, Bombay.

Dr. G. SANKARAN, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Coopted members.

Dr. J. B. GRANT, Director, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. W. R. AYKROYD, Director, Nutrition Research Laboratories, I. R. F. A., Coonoor.

Dr. JEAN M. ORKNEY, W.M.S., Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi.

Composition of Advisory Committee for the year 1943.

1. *Malaria.*

Brigadier G. COVELL (*Chairman*).
Dr. ANANTASWAMI RAO
Rai Bahadur Dr. A. C. BANERJEA
Bt.-Colonel Sir RAM NATH CHOPRA
Mr. K. SUBRAHMANYAN
Dr. D. K. VISWANATHAN
Major JASWANT SINGH (*Secretary*)

2. *Nutrition.*

Lieut.-Colonel E. COTTER (*Chairman*)
Professor S. P. AGHARKAR
Dr. K. P. BASU
Dr. N. M. BASU
Dr. W. BURNS
Khan Bahadur Dr. A. H. BUTT
Dr. B. C. GUHA
Dr. V. N. PATWARDHAN
Dr. G. SANKARAN
Mr. J. SARGENT
Mr. F. WARE
Dr. W. R. AYKROYD (*Secretary*)

3. *Plague.*

Lieut.-Colonel S. S. SOKHEY (*Chairman*)
Dr. K. A. GANDHI
Dr. V. R. KHANOLKAR
Dr. C. G. PANDIT
Major-General Sir JOHN TAYLOR (*Secretary*)

The Board held two Meetings during the year, the first on the 7th and the second on the 8th December, 1942.

At its meeting on the 7th December, 1942, the Board confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers, and also decided on the cooption of experts for its meeting on the 8th December, 1942. On that date the Board considered in detail the programme of research for the year 1943-44. The programme of researches recommended by the Board is given on pages 129–132 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1942.

The researches, which were carried out during the year under report, and which were recommended by the Board at its meeting on the 16th and 17th December, 1941, were approved by the Governing Body of the Association at their meeting held in the Imperial Secretariat, New Delhi, on the 14th March, 1942.

CHOLERA

1. Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta.

TREATMENT OF CHOLERA WITH SULPHANILYLGUANIDINE.

(a) *In vitro experiment*—Vibrio cholerae was inoculated into 4 c.c. of nutrient broth to which one gramme of sulphanilylguanidine was added previously. This was plated out on bile-salt agar medium at different times and the results are as follows :—

Statim plating shows growth of vibrios						—plenty.
18 hours	„	„	„	„	„	— fair in number.
42 hours	„	„	„	„	„	— few in number and a few other colonies.
60 hours	„	„	no growth of vibrios but plenty of other bacteria.			

Controls without sulphanilylguanidine show growth of vibrios in all the plates.

Controls of sulphanilylguanidine without the culture show growth of other bacteria.

Considering that the contaminants of the drug might have overgrown the vibrios, sulphanilylguanidine was added to 4 c.c. of nutrient broth and autoclaved. Then the culture was inoculated and plated out on bile-salt agar medium at different times with the following results :—

Plating statim, at 4 hours, 24 hours, 48 hours, 72 hours and 96 hours	.. Shows growth of many colonies of v. cholerae.
Control without the culture — plating at the above periods	.. Shows no growth of any organisms.

(b) *In vivo experiment*—Guinea-pigs were infected with 12 c.c. of broth cultures of live vibrio cholerae by mouth and subsequently the drug was administered in 0.1 gm. doses intragastrically statim, at 1 hour, 2 hours and 4 hours. In another group the drug was mixed with the culture and given by mouth. Preliminary results show a definite effect of the drug and the work is in progress.

(c) *Treatment of cholera cases*—This drug was administered to 77 patients on admission to the cholera ward for 24 hours, 31 of these were Ogawa type, 19 Inaba type, 1 Nag, and the rest 26 were bacteriologically negative. Of

134355

the 77 cases treated with sulphanilylguanidine 35 were admitted with suppression of urine. Of these 25 passed urine within 24 hours, 8 within 48 hours and 2 within 72 hours. There was no death.

Among 76 control cases, 29 were Ogawa type, 20 Inaba type, 6 Nag, and the rest 21 were bacteriologically negative. Of 76 control cases, 36 were admitted with suppression of urine. Out of these, 16 passed urine within 24 hours, 4 within 48 hours and 4 within 72 hours. There were 5 or 7 per cent deaths, 7 developed pre-uraemic symptoms and were treated with sulphanilylguanidine. They were cured.

The dose of the drug :—5 grammes of the drug was given statim on admission and 2.5 grammes every four hours until 20 grammes in all had been given.

The thirty cases treated with sulphanilylguanidine had on an average 3 pints of intravenous saline per head whereas the control cases had 5 pints of intravenous saline.

Among the patients treated with sulphanilylguanidine vibrios were found in 14 and of these vibrios were recovered for 1, 2 and 3 days from 7, 4 and 3 patients, respectively, whereas among 20 control cases 13, 2, and 5 patients excreted vibrios for 1, 2 and 3 days, respectively.

Treatment of cholera with sulphasuxidine.

Only 4 cases were treated with this drug with no death, whereas there was also no death in 2 control cases. The test will be continued.

Treatment of cholera with green dye.

(a) *In vitro experiment*—All agglutinating vibrios (including Inaba and Ogawa sub-types and El Tor are highly sensitive to green dye such as brilliant or malacite green. A dilution of 1/100,000 is bacteriocidal to broth culture. Complete sterilization occurs in 12–18 hours which is usually indicated by the persistence of green colour. Most of the non-agglutinating vibrios discharge the colour of the dye by their growth and are dye resistant. Some non-agglutinating vibrios isolated from cholera stools were sensitive to dye like agglutinating vibrios. These were generally more toxic to guinea-pigs.

(b) *Treatment of cases*—Vibrios usually disappear from the stool in 2-3 days on administration of 1/2500 dilution of brilliant green sweetened with 2 per cent cane sugar and flavoured with a little spt. menth. pip., whereas in cases not treated with the dye vibrios disappeared from the stool in from 4 to 7 days.

Pyrogen free distilled water.

In connection with the treatment of cholera cases with pyrogen free saline it was difficult to find any organic matter in 10 c.c. of tap water. This quantity when used after proper sterilization and being made into normal saline, for injection into normal rabbits did not produce an appreciable rise of temperature. Later 500 c.c. of tap water was concentrated by boiling to 30 c.c. Presence of organic matter was demonstrated in this concentrated water. Ten c.c. of this when made into normal saline and injected intravenously into normal rabbits gave rise to a temperature of over 1. 1°C. on the average, in spite of some of the pyrogenic substances in all probability having been destroyed by prolonged boiling. The normal variation was 0.5°C. The work which was aimed at finding a practical way of providing pyrogen free water in the quantities required for treating cholera patients, is in progress.

ISOLATION OF VIBRIOS.

(a) *Cholera stools*—Examination of cholera stool was carried out by candle-boric-peptone-water method, by direct plating on the bile-salt agar and Wilson and Blair's solid media respectively. Vibrios could be isolated in 188 out of 233 samples of stools (81 per cent) by candle method, in 137 or 59 per cent by direct plating on bile-salt agar, and in 136 or 58 per cent on Wilson Blair's media. Pure or almost pure cultures were often obtained from the boric-peptone-water outside the candle. The only disadvantage was that sometimes the growth of vibrios was delayed for 48 hours.

(b) *Hooghly water*—Eighty-three samples of water were passed through kieselguhr and filter papers and then examined by two methods, of these 9 samples were positive by direct plating on bile-salt agar media and 52 samples were positive by candle-boric-peptone-water method. Six hundred and forty-four samples of Hooghly water collected in different places from day to day were then examined by candle method only. Nag vibrios were isolated from the majority of these samples. Inaba and Ogawa sub-types were isolated from 15 samples only. (This is carried out by Dr. Panja himself).

BACTERIOLOGICAL STUDIES OF V. CHOLERAE.

(a) Eightyone colonies of vibrios isolated from 23 clinical cholera cases were split up and found to be of the same original type. It appears therefore that except in rare instances, as previously found, a colony of vibrios obtained by plating can be regarded as a pure colony.

(b) Vibrios cholerae were added to a few samples of Hooghly water and could not be isolated from the mixture after 3–4 days ; but they could be isolated up to the 3rd week when they were added to the same Hooghly water filtered through L_3 candle. Artificial mixtures of AG and Nag vibrios in peptone water and nutrient agar slopes show that Nag vibrios overgrow the AG vibrios causing the latter to disappear ultimately from the mixture.

(c) *Invasiveness and toxicity test of vibrios*—Nag vibrios from Hooghly water and Nag and AG vibrios from clinical cholera cases were tested for invasiveness by injecting them subcutaneously into guinea-pigs, and culturing the blood of these animals for vibrios every 15 minutes up to 4 hours and intestinal contents and blood again after death. For toxicity tests, guinea-pigs were also fed with heavy doses of live cultures of vibrios, and rabbits were injected intravenously with the vibrios. Post-mortem, intestines and their contents were examined. The work is in progress.

(d) *Skin test*—Killed saline suspensions of vibrios, B. coli, and B. flexneri gave rise to erythema and oedema at the site of intradermal injection in cholera cases as well in healthy individuals though to a less extent in the latter. Endo-toxin obtained by alternate freezing and thawing was tried. A diluted solution gave more marked reaction in most cholera cases than in normal persons. This is in progress.

(e) *Antigenic structure*—All the Nag strains isolated from the beginning of the disease are agglutinated by the serum raised against one of them, indicating that these are apparently all of the same antigenic structure. But those isolated in the later stage of the disease caused by AG vibrios are not always serologically identical.

MISCELLANEOUS STUDIES.

(a) *Cholera convalescent sera*—Guinea-pigs were injected intraperitoneally with living cultures and later on with the serum of convalescent cholera patients, subcutaneously in one group and intraperitoneally in another group. There was no protection. Cholera culture and convalescent serum were mixed together and injected intraperitoneally into guinea-pigs. There was no death, probably because of the bacteriolytic action of serum and the consequent arrest of growth of vibrios.

(b) *Cholera vaccine*—Kasauli cholera vaccine and cholera vaccines prepared in this laboratory after being boiled for 1 hour still afforded complete protection to guinea-pigs immunised by three injections at weekly intervals against 5 minimal lethal doses.

(c) During the experiments of candle method, water was concentrated by filtration and it has been found that unconcentrated water yielded better results than concentrated water in the isolation of vibrios. Out of 66 samples, 59 samples were positive by direct (unconcentrated) method and 47 samples were positive by filtration (concentrated) method.

(d) In the toxicity test and *in vivo* experiment with sulphanilylguanidine, the guinea-pigs were fed by the following method. A long intravenous saline needle was blunted at the end. This was introduced into the mouth of the guinea-pig which was held open by a pair of forceps and then by slight push backwards and downwards the needle used to pass through the pharynx to the oesophagus. A measured quantity of the drug was then administered by a syringe.

2. Enquiry to determine the endemic foci of cholera in certain parts of Bengal and Madras Presidencies under Dr. K. C. K. E. Raja, Offg. Deputy Public Health Commissioner with the Government of India.

A study of cholera mortality figures in Madras Presidency in 1929 led Sir Alexander Russell (who was then Lieut.-Colonel A. J. H. Russell, I.M.S., and Director of Public Health, Madras) to the conclusion that the disease was endemic in Tanjore and possibly in the neighbouring districts of Trichinopoly and South Arcot. Russell's investigation covered the period from 1896 to 1925. In view of the relatively smaller incidence of cholera in more recent years the present enquiry has been undertaken in order to re-investigate the endemic status of these three districts. This study is based on the weekly cholera mortality figures for the period, 1927-1941.

In order to compare the incidence of cholera during the two periods 1896-1925 and 1927-1941 the following table has been prepared. The figures in column 3 have been reproduced from table 1 on page 99 of Russell's book entitled "A geographical survey of cholera in the Madras Presidency" and they relate to the period 1896-1925. Those in column 4 relate to the period 1927-1941.

A comparison of the incidence of cholera in the different districts of Madras Presidency during the two periods, 1896-1925 and 1927-1941 based on monthly cholera mortality figures.

Serial No.	Name of district.	Percentage of months free (from Russell's table) for the period 1896-25.	Percentage of months free for the period 1927-1941.
1.	Vizagapatam ..	31.4	32.8
2.	Godavari ..	33.3	40.6*
3.	Bellary ..	36.4	44.4
4.	Anantapur ..	38.6	51.7
5.	Kurnool ..	48.3	47.2
6.	Cuddapah ..	30.8	45.6
7.	Kistna and	28.8	41.7
8.	Guntur ..		47.8
9.	Nellore ..	26.1	37.2
10.	Chingleput ..	18.6	28.9
11.	North Arcot and	13.1	27.8
12.	Chittoor ..		41.1
13.	Salem ..	11.1	33.9
14.	Coimbatore ..	19.4	30.6
15.	Madura and	14.7	30.6
16.	Ramnad ..		29.4
17.	Tinnevelly ..	15.0	28.9
18.	Trichinopoly ..	8.6	17.8
19.	Tanjore ..	3.1	15.6
20.	South Arcot ..	6.7	17.2
21.	Malabar ..	14.7	53.3
22.	South Kanara ..	38.8	65.0

* Godavari has now been split into two districts, East and West Godavari and the figure for both is 40.6.

It will be seen that, in certain cases, only combined figures for two districts have been shown in column 3. This is because the bifurcation of the territory concerned took place at some time during the period 1896-1925. From columns 3 and 4 it will be seen that, with the excepton of Kurnool, the incidence of the disease as judged by the absence of cholera mortality figures for individual months, has been less during 1927-41 in comparison with 1896-1925. The decrease in respect of the districts of Tanjore, Trichinopoly and South Arcot is striking. Russell considered that for the period 1896-1925, Tanjore need alone be classed as definitely endemic and the other two as possibly endemic. Under the circumstances, for the period 1927-1941, it is clear that the present enquiry into the endemicity of cholera in Madras Presidency need not be extended beyond these three districts.

The incidence of cholera during the two periods may also be examined from another point of view. Cholera deaths during a period of thirty years from 1912 to 1941 are shown below. The figures for individual periods of six years have been made into percentages of the total for 1912-1941 for each area and are shown in brackets.

Place	Total cholera deaths during 1912-41	1912–17	1918–23	1924–29	1930–35	1936–41
Tanjore ..	107,462	31,375 (29.2)	18,940 (17.6)	36,970 (34.4)	10,193 (9.5)	9,984 (9.3)
Trichinopoly ..	64,004	16,418 (25.6)	10,690 (16.7)	25,350 (39.6)	7,075 (11.1)	4,471 (7.0)
South Arcot ..	76,430	21,327 (27.9)	15,177 (19.9)	14,624 (19.1)	18,854 (24.7)	6,448 (8.4)
Rest of Madras Presidency	787,656	235,328 (29.9)	250,592 (31.8)	163,106 (20.7)	75,599 (9.6)	63,031 (8.0)
Totals for province ..	1,035,552	304,448 (29.4)	295,399 (28.5)	240,050 (23.2)	111,721 (10.8)	83,934 (8.1)

It will be seen that, for the province as a whole, there has been a steady decline in cholera mortality through this period of 30 years. Of the three districts for which figures have been given separately, Tanjore and Trichinopoly had a rise in the figure during the six yearly period 1924-29 compared with the two previous periods, while in South Arcot a rise is noted in the period 1930-35. But, broadly speaking, the mortality from cholera seems to have decreased from 1930 to 1941 to such an extent as to suggest that there might have been introduced some factor or factors tending to produce a lowered incidence of the disease.

For a more detailed study of the decline of cholera during the periods 1912-26 and 1927-41 respectively, linear trends were investigated separately in respect of the three districts of Tanjore, Trichinopoly and South Arcot and for the remaining portion of the province as a single unit. During 1912-26 a decrease in cholera incidence was indicated for all the areas by the downward slope of the straight lines fitted to the figures, but a statistically significant fall was not demonstrated in any of them. On the other hand, for the period 1927-41, the decline was shown to be statistically significant for all except South Arcot district. This again suggests that, within more recent years, the factors favouring the epidemic prevalence of the disease might have undergone a change.

A study of the figures for Tanjore district may also be made. On page 101 of his book "A Geographical Survey of Cholera in Madras Presidency" Russell has given a table indicating the extent to which individual taluks of Tanjore showed freedom from cholera as judged by mortality figures during a period of 156 months (1913-25). The table is reproduced on the next page:—

Tanjore District: 1913-25.

Name of Taluk	Total number of months in which the taluk was free during the 13 years from 1913 to 1925 (156 months).	Percentage of months free.	The maximum period of freedom from cholera at a time.	Total number of such periods.	Number of times the taluk was free for 3 months or more at a stretch but less than 6 months.	No. of times the taluk was free for 6 months or more at a stretch.
(1)	(2)	(3)	(4)	(5)	(6)	(7)
			months			
Tanjore ..	31	19.9	6	1	2	1
Papanasam ..	50	32.1	6	1	7	1
Kumbakonam ..	50	32.1	7	1	4	1
Mayavaram ..	48	30.8	10	1	4	2
Shiyali ..	56	35.9	8	1	5	4
Nannilam ..	47	30.1	17	1	5	1
Negapatam ..	58	37.2	15	1	4	3
Mannargudi ..	50	32.1	17	1	4	2
Tirutturaipundi ..	55	35.3	9	1	5	4
Pattukottai ..	52	33.3	12	1	6	1
Arantangi ..	90	37.7	22	1	5	6

A similar table has been prepared for the years 1927-1941, a period of 15 years or 180 months. As monthly cholera mortality figures were not available for all the 15 years, the weekly figures supplied by the Director of Public Health, Madras, were grouped into 4-weekly totals for the preparation of this table.

Tanjore District: 1927-41.

Serial Number.	Name of taluks in the Tanjore district.	Total No. of 4-weekly periods in which the taluk was free during the 15 years from 1925 to 1941 (195 4-weekly periods).	Percentage of four-weekly periods free.	Maximum No. of four-weekly periods of freedom from cholera.	Total number of such periods.	Number of times the taluk was free.	
						For 13 weeks (3 months) or more at a stretch but less than six months.	For 26 weeks (6 months) or more at a stretch.
(1)		(2)	(3)	(4)	(5)	(6)	(7)
1.	Tanjore ..	145	74.4	29	1	3	10
2.	Papanasam ..	113	57.9	25	1	10	4
3.	Kumbakonam ..	106	54.4	23	1	3	6
4.	Mayavaram ..	114	58.5	21	1	9	6
5.	Shiyali ..	135	69.2	12	1	7	8
6.	Nannilam ..	107	54.9	25	1	4	5
7.	Negapatam ..	123	63.1	58	1	4	4
8.	Mannargudi ..	141	72.3	47	1	3	7
9.	Tirutturaipundi ..	111	56.9	22	1	8	4
10.	Pattukottai ..	120	61.6	20	1	5	7
11.	Arantangi ..	139	71.3	41	1	6	7

A comparison of columns 3, 4, 6 and 7 in the two tables gives some indication of the extent to which a lessening of the incidence of cholera mortality has taken place in Tanjore district.

It has been pointed out by Russell that continuity of cholera infection in the three districts of Tanjore, Trichinopoly and South Arcot seems to have been maintained as the result of a combination of irrigated and non-irrigated areas with variations in their seasonal cholera incidence. For studying the endemicity of cholera the area under investigation should be homogeneous with respect to its cholera history. What is meant is that the component parts of the area should not show marked variations in the incidence of the disease either from year to year or from season to season within each year. It is only when the absence of such variations can be established and when continuity of infection can be shown to exist that there is reason to believe that the factors favouring the propagation of cholera continue to be present in the area throughout the year. The first step in the present investigation was therefore the examination of the figures for cholera mortality in individual taluks of these three districts, which are the smallest units of administration for which figures are available for the period, 1927-41. There are 26 taluks in all in the three districts. By the method of analysis of variance the annual and seasonal variabilities of cholera incidence within each taluk were separated. Next contiguous taluks were compared with respect to these characteristics as well as the mean incidence of cholera. As a result of these studies an area consisting of eleven contiguous taluks (six in Tanjore, three in Trichinopoly and two in South Arcot) have been shown to form one homogeneous cholera district. The remaining fifteen taluks differ from one another to such an extent that any two of them cannot be combined.

It is now proposed to investigate the question of endemicity in this homogeneous cholera area and in each of the remaining fifteen taluks of the three districts.

MALARIA.

3. Malaria Institute of India (Research Section), Kasauli/Delhi.

The research activities of the Institute have again been severely curtailed owing to abnormal conditions brought about by the war. The post of Malaria Research Officer has remained unfilled, and a large proportion of the work of the staff has been devoted to instructional courses in malariology for officers deputed by the War Department. Six of these courses have been held during the year at which 152 officers have been trained, including 9 Chinese and 4 members of the U. S. A. Medical Corps.

Publications.

Journal of the Malaria Institute of India—Two numbers containing 16 papers and 3 abstracts have been published during the year.

Scientific material sent to other workers.

Strains of monkey malaria have been supplied to the Calcutta School of Tropical Medicine and to the National Institute of Health, Chungking. Stained blood films illustrating the various stages of the malaria parasite, preparations of infected mosquito guts and glands and mounted specimens of mosquitoes and larvae have been supplied to workers in various parts of India.

Testing of insecticides and sprayers.

An extensive series of experiments have been conducted to test the mosquitocidal value of a number of insecticides. The efficiency of certain insecticides against the body louse has been investigated. Observations have also been carried out regarding the action of storage on the lethal properties of insecticides. The effect on the stability of pyrethrins of various emulsions and emulsifiers has been tested with the object of rendering unnecessary the use of kerosene oil as a vehicle for pyrethrum extracts. A suitable emulsifier for this purpose has now been discovered.

Various types of sprayer for use with insecticides and larvicides have been tested. The standard type of hand sprayer, originally designed at the Institute, has been modified in certain respects with a resultant increase in efficiency.

Testing of repellents.

A large number of experiments have been carried out with various types of anti mosquito cream. As a result of this work, a gum jelly with pyrethrum extract as the active principle has recently been recommended for use by the army. This has the great advantage of being non-greasy and therefore pleasant to use. It is at present undergoing field trials before final adoption.

Entomological researches.

Colonies of *A. stephensi* (type form) and *Aedes aegypti* (Karachi strain) have been maintained in the laboratory. The bionomics of these colonies have been studied intensively.

Numerous collections of mosquitoes received from workers in different parts of India have been identified.

Field researches.

(a) *Delhi*—Epidemiological studies have been carried out in certain villages. The occurrence of a regional epidemic affecting Delhi Province has provided a rich field for investigation, and a large amount of valuable data has been collected for analysis. The antimalaria operations in Delhi have been directed and supervised as in previous years.

(b) *North Kanara*—Epidemiological observations have been carried out in a number of localities by a research unit whose headquarters are located at Sirsi. A comprehensive malaria survey is being conducted in this area comprising routine spleen and blood examinations, mosquito dissections and a study of the bionomics of the local anopheline species. From the data hitherto collected it appears that *A. fluviatilis* is the principal, if not the only, vector of malaria in this area.

Laboratory researches.

(1) A number of monkey malaria strains have been maintained. (2) High titre anti human serum for determining the source of mosquito blood meals by the precipitin test has been prepared. In preliminary experiments, the intraperitoneal injection of dried human plasma has proved more successful than normal human serum for preparing antisera from fowls. Several hundred mosquito blood meals from various parts of India have been tested. (3) A number of samples of strains for use in the diagnosis of malaria parasites in blood films have been tested. (4) Certain drugs have been tested as to their effect on monkey malaria. In no case have the results indicated that the drug would be of any value in the treatment of human malaria. (5) Approximately 5,000 blood films have been examined for parasites of human malaria.

Southern India Branch.

(1) A series of 1,946 anopheline dissections including 13 species from localities at 5,600 to 7,000 feet elevation has been carried out with negative results. At a tea estate at Kullakamby (5,030 feet), however, where a malaria epidemic was in progress, 7 specimens of *A. fluviatilis* were found infected out of 19 dissected.

(2) A number of infectivity experiments with *P. knowlesi* was carried out. From one batch of 28 specimens of *Armigeres obturbans* 3 became infected (salivary glands). Sporozoites in saline suspension from one of these inoculated intravenously into a clean *sinicus* monkey produced an infection after an incubation period of 16 days. Sub-inoculation from this monkey was successful. Sporozoites from the other two infected mosquitoes failed to produce an infection.

(3) Bacteria-free first instar larvae of *A. stephensi* (type) and *Aedes albopictus* were supplied to the Director, Nutrition Research Laboratories, Coonoor, for certain experiments.

(4) Studies on the mosquito fauna of the Nilgiris are in progress. Thirty-one species of culicines, belonging to 12 different subgenera, have been identified. The larvae of 10 of these species have not previously been recorded in India.

(5) Four species of culicine mosquitoes have been infected with parasites of avian malaria up to the sporozoite stage, one with *P. gallinaceum* and 3 with *P. praecox*. So far as is known, these observations have not been recorded elsewhere.

(6) Special studies have been made on the blood picture of fowls infected with *P. gallinaceum* with special reference to the effect of phenylhydrazine hydrochloride.

(7) Strains of *P. knowlesi* and *P. gallinaceum* and laboratory colonies of *A. stephensi* (type) and *Aedes albopictus* have been maintained.

(8) Observations have been carried out on the egg patterns of wild caught *A. fluviatilis*.

4. Enquiry into the adult habits of *A. varuna* under Major R. Senior White, Malariologist, B. N. Railway, Calcutta.

The enquiry was commenced in 1940. It was resumed in July 1942, at the same place, an isolated gang hut in the forests of Khairagarh State, Central Provinces.

The procedure followed was the same as has been described in the preliminary paper published in the Journal of the Malaria Institute of India iv (1) 57, but this year each room was furnished with a padlock to which the insect collector had a duplicate key, so that the error due to the occasional non-searching of locked rooms, that occurred in 1940, was avoided. There are now 13 rooms at the site. A few cattle are now kept by the gang, actually in their boarded up verandahs.

Results up to October 25th, 1942, are shown in the following table. Owing to the small numbers (57) again caught in the trap net, both at 2 a.m. and 6 a.m., the figures for the two catches have been combined. The 2. a.m., catch is 37% of the combined trap net catches in the *funestus* group. This catch alone is certainly attempting to bite. It included no infected specimens.

	July			August			September			October to 25th.		
	No.	Oocyst Rate	Sporozoite Rate	No.	Oocyst Rate	Sporozoite Rate	No.	Oocyst Rate	Sporozoite Rate	No.	Oocyst Rate	Sporozoite Rate
I. In trap net at 2 a.m. and 6 a.m.												
A. fluviatilis	0		..	0	..	..	17	5.9	0	37	0	0
A. varuna	0	..	..	0	..	..	3	0	0	0	..	..
% of *funestus*-group in catch	0	..	..	0	..	..	8.0	..	..	31.4	..	..
II. In 13 rooms morning												
A. fluviatilis	6	16.7	0	87	5.7	4.9	512	3.1	2.3	798	3.9	2.5
A. varuna	0	..	..	0	..	..	46	4.3	4.3	75	4.0	0
% of *funestus*-group in catch	0.2	..	..	2.0	..	..	15.2	..	..	38.5	..	..

The *varuna* : *fluviatilis* ratio has again been low, 1 : 12. Another comparative catch at Chandsurat village, made on the same day as those recorded for 1939 and 1940, showed a 1:8 ratio. Evidently the spot selected for the experiment was not favourable to *varuna* breeding. As no previous surveys of the spot existed this could not have been foreseen and was unexpeeted. Once again therefore more information has been forthcoming on the habits of *A. fluviatilis* than of *A. varuna*.

Though catching has been as complete as possible, each room being gone over twice, it is not claimed that complete emptying has been achieved. A few mosquitos probably avoided detection. However for *A. varuna*, sporozoites have been found twice, and this species locally appears to be entirely a house rester. The case is otherwise in *fluviatilis*, in which not only have sporozoites been frequent, but the ratio of gland to gut infections is higher than normal. If, as is believed, malaria transmission in this area is a closed circuit, it provides a definite indication that much *fluviatilis* resting in the Satpura ranges occurs out of doors, and therefore complete malaria prevention by spraying cannot possibly be achieved.

That part of the enquiry conducted by the railway staff around Vizagapatam has been completed. Whereas *A. varuna* there is very rare as a house-resting adult, the staff captured and dissected from 1940 to 1942 over eleven thousand specimens by using special trap huts of palmyra thatch, baited by men and by buffaloes. The sporozoite rate was found to be 0.02% only as compared with 3.7% in general house collecting in the Satpuras, and 1.1% in both seasons at the experimental site. This indicates that the Northern Circars Plains species is biologically distinct, and as a vector is negligible, being found infected only when forced on to man. The Malaria Institute has carried out precipitin reaction on 858 specimens of the Vizagapatam material, with the following results :

Caught in	walled cattle sheds, androphilic index	.. 4.9
,, ,,	buffalo baited trap hut, ,, ,, ,,	.. 8.3
,, ,,	open sided cattle shed, ,, ,, ,,	.. 27.4
,, ,,	human baited trap hut, ,, ,, ,,	.. 58.5

Apparently these two are not the only biologically distinct races. At Chatikona in the Jeypore hills *A. varuna* has a 6.1% sporozoite rate, and the androphilic index (house catches, numbers small) is 100. In Singhbhum the sporozoite rate is 1.5% whilst catches made for precipitin tests vary from 55.5% on man in house catches to 100% bovine on 26 specimens caught resting under culverts, as well as showing that in this district, where alone such searches have been made, there is much out of door resting.

The maxillary index of *varuna* from the Jeypore hills shows no statistical difference from that of specimens from Bengal or Vizagapatam already recorded. It is hoped to complete egg measurement comparisons before the end of the year.

Though it is thus obvious that the study of *A. varuna* habits as an adult has been no more than begun, it is not proposed to ask for a further grant for this. Further investigations can best be pursued by means of extensive precipitin tests.

NUTRITION.

5. Nutrition researches under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, I. R. F. A., Coonoor.

Progress has been made during the year in various fields of research, which have included the following :

The composition of foodstuffs.

Further work has been done on the content of foods in various members of the vitamin B complex, using methods and improved techniques evolved in the Laboratories. The effect of washing and cooking on the vitamin B_1 content of raw and parboiled milled rice has been studied, employing an improved thiochrome method. It was found that raw milled rice lost about 60 per cent of its vitamin B_1 content during the process of washing and about 20 per cent on cooking. Parboiled milled rice, on the other hand, lost a much smaller percentage. The vitamin B_1 content of washed and cooked parboiled milled rice is about 4 times that of washed and cooked raw milled rice.

This investigation further clarifies the observation made by Aykroyd *et al* (1940)* that beriberi as a serious public health problem occurs in places where raw milled rice is consumed by the poor, e g. the Northern Circars district of the Madras Presidency, Malaya, China, Java and Japan, and is rare where parboiled milled rice is the chief staple. It also confirms the original hypothesis of Aykroyd (1932)† that ' when rice is parboiled the endosperm absorbs vitamin B_1 at the expense of the germ and pericarp and milling does not remove the vitamin '. In raw rice most of the vitamin is located at the surface of the grain and is readily washed away, whereas in parboiled rice it is located more deeply and is less easily dissolved out by the water used in washing. Parboiled milled rice has thus two advantages over raw milled rice : (a) it has a higher vitamin B_1 content and (b) the vitamin B_1 present in parboiled rice is more difficult to wash out. Both are of importance in producing the almost complete immunity of the consumer of parboiled milled rice to beriberi.

A detailed study of the vitamin C in germinated pulses was carried out. The fact that pulses and other grains, on germination, synthesise vitamin C has been known for a long time, but since germinated pulses may be of practical value as anti-scorbutic agents under war conditions, it was felt that further data on this subject would be of immediate interest. Of the pulses studied, green gram (*Phaseolus radiatus*) synthesised the largest amount of vitamin C on germination and soya bean the smallest. Vitamin C content reaches a peak about 30 hours after germination has begun, after which it remains constant for 4–5 days. Germinated pulses lose a considerable amount of vitamin C on cooking, and in practice should be consumed raw or after a minimum amount of cooking. During this investigation unexpectedly high values for vitamin C (10–20 mg./100g.) were obtained by chemical methods in samples of dry ungerminated Bengal gram (*Cicer arietinum*). This result was confirmed by biological tests on guinea pigs, and is of practical interest and importance. Bengal gram is a common article of diet in most parts of India and the fact that it contains

* "The Rice Problem in India" by Aykroyd, W. R., Krishnan, B. G., Passmore, R., and R. Sundararajan, A. R. *Ind. Med. Res. Memoir* No. 32, January 1940.

† Aykroyd, W. R. (1932) *Jour. Hyg.*, 30, p. 357.

vitamin C in the dry ungerminated state may do something to explain the relative rarity of scurvy even when intake of fresh fruits and vegetables is very small. It was found that Bengal gram contains an alkali-stable factor which promotes growth in guinea pigs ; the nature of this factor is at present unknown.

An investigation of vitamin C and carotene in dehydrated vegetables and fruits was undertaken. Dehydrated vegetables are now being produced on a large scale for military purposes, not only in India but also in Great Britain and the United States of America. The subject is therefore of immediate practical interest. The losses of vitamin C on dehydration are always considerable, but some vegetables remain fairly rich in vitamin C after dehydration. The preliminary " blanching " or " scalding " of vegetables before dehydration has a protective effect on vitamin C, since it destroys oxidising enzymes. An important point is the stability of vitamin C in the dehydrated vegetable, which will usually be consumed several months after manufacture. Tests carried out on material immediately after dehydration give little indication of its antiscorbutic value under war conditions. Commercially dehydrated (steam-blanched) vegetables were kept for several months in an incubator at 37°C. in sealed (not exhausted) tins. The tins, containing samples from a consignment of each of the vegetables processed on one particular day, were opened at intervals for testing. It was found that dehydrated cabbage, cauliflower, and knol-khol lost 50 per cent of the ascorbic acid originally present in the dehydrated material in 12 weeks. In a sample of dehydrated potato stored at room temperature in an unsealed closed tin the loss was only 10 per cent in 12 weeks. Vitamin C appears to be more stable in material which remains *crisp*. The stability of vitamin C in dehydrated vegetables in relation to conditions of packing and storage, the method of " scalding ", time of dehydration, etc., obviously requires further investigation.

The reconstitution and cooking of dehyrated vegetables lead to very considerable loss of vitamin C. The general conclusion seems to be that certain dehydrated vegetables, after storage for 2–3 months and ordinary cooking, may supply small but not altogether negligible amounts of the vitamin. They cannot be regarded as reliable antiscorbutics, because vitamin C is not stable in the dehydrated material and the amount left varies with the period of storage.

Dried guavas (*Psidium guyava*) have been recommended by workers in South Africa as a practicable antiscorbutic.* By using a suitable method of dehydration, dried guava pulp with a value of 22 mg./g. of vitamin C was obtained —a very potent product. Unfortunately it was found that vitamin C is very unstable in this material, a loss of 30 per cent in a few days being observed. In the circumstances the use of dehydrated guavas as an antiscorbutic can scarcely be recommended.

Carotene (pro-vitamin A) is affected to a smaller extent than vitamin C by the dehydration process, and losses on reconstitution and cooking are relatively small. Carotene in dehydrated vegetables appears to be fairly stable on storage.

The discovery made by Roumanian workers that immature walnuts (*Juglans regia*) are a very rich source of vitamin C was confirmed. A value of nearly 16 milligrammes per gramme of fresh material was observed in the case of certain samples. The vitamin is present mainly in the pulp. As the fruit ripens and the nut is formed, the vitamin disappears. Dried walnut pulp with the remarkably high value of 106 mg. of vitamin C per gramme was prepared. In spite of the high vitamin C value of tender walnuts, their use as an antiscorbutic in the fresh or dehydrated form does not appear to be very practicable.

* Goldberg, L., and Levy, L. (1941) *Nature*, 148, p. 286.

The tender fruit is very bitter, and the dried product is so objectionable in taste that it could scarcely be swallowed. Further the vitamin is not stable on storage. Tender walnuts, stored in the refrigerator, lost 30 per cent of the vitamin in 3 weeks and the loss in the dehydrated material on storage at room temperature for a similar period was 68 per cent. It might be possible to devise methods of preparation (e.g. pickling) which would to some extent disguise the bitter taste, or alternatively, the vitamin might be extracted from the fruit by suitable means. But there are the practical disadvantages that the fruit would have to be obtained and processed at a certain brief stage of immaturity, the ripe fruit being devoid of the vitamin, and that fully mature walnuts are a valuable commercial crop.

The nutritive value of soya bean has been investigated by experiments on rats, following the methods suggested by the Soya Bean Sub-Committee of the Nutrition Advisory Committee. The object of the investigation is to compare the value of soya bean with that of common Indian pulses, with a view to deciding whether the production and consumption of soya bean is worth encouraging in India.*

Numerous samples of fish liver oil have been tested for vitamin A by colorimetric and spectrophotometric tests. Shark liver oil is now being produced at various centres on the West Coast of India, to meet the demand created by the shortage of cod liver oil. A useful local industry is being developed, which, it is to be hoped, will continue to exist after the war when supplies of cod liver oil become once more available.

Miscellaneous investigations.

It has been shown that rabbits, like rats, are able to synthesise nicotinic acid. In the course of this investigation current methods for the estimation of trigonelline were modified and methods of determining nicotinic acid in urine improved.

Further work has been carried out on the protective effect of calcium salts on fluorine poisoning in rats. Calcium carbonate, chloride, gluconate, and oxide have been found to exert such an effect.

Dietary experiments have been carried out using rice moth larvae (*Corcyra cephalonica Staint*). These larvae can synthesise vitamin C, but require most of the known constituents of the vitamin B complex for satisfactory growth. When fed on a vitamin B_1-deficient diet they accumulate pyruvic acid, which disappears when the vitamin is supplied ; this fact is being used as the basis of a test for very small quantities of vitamin B_1 e.g. those present in blood. The protein requirements of this species have also been studied. The fact that the dietary requirements of insect larvae resemble so closely those of mammals including man is of great biological interest.

Clinical and field investigations.

In last year's report reference was made to the peak in infantile mortality in the 3rd and 4th month of life which occurs in the beriberi area in Northern Circars. The suggestion was put forward that this mortality was due to infantile beriberi. In order to throw further light on the problem, a field enquiry on infantile beriberi has been instituted in Cocanada, after some unavoidable delay due to war conditions. Within a few months numerous cases of acute vitamin B_1

* Research along similar lines is being carried out simultaneously in 3 other laboratories.

deficiency in infants have been observed and successfully treated by injections of thiamine. The clinical features of the condition do not completely correspond with the classical text-book picture of infantile beriberi. A previously healthy breast-fed infant is seized with attacks of pain and screaming fits. It shows some diarrhoea and may vomit its feeds. There is often excoriation of the skin in the neighbourhood of the anus and soreness of the lips and mouth. The infant is obviously ill and unless treatment is given the screaming attacks and pain may continue until it becomes weak and eventually dies. Aphonia is often a feature of severe cases, but general oedema has not been observed. The injection of thiamine produces an almost immediate effect.

The mother usually, but not always, shows evidence of mild beriberi—e.g. some pain and tingling in the extremities. Her diet consists mainly of raw machine-milled rice. The disease does not appear to recur in the infant after treatment with thiamine if the mother can be persuaded to eat home-pounded rice or millet, or is given yeast regularly.

There seems little doubt that the high mortality in the beriberi area occurring in infants in the 3rd and 4th month of life is due to vitamin B_1 deficiency.

Vitamin B_1 can now be cheaply manufactured on a large scale. The latest price quoted by a firm in England is about Re. 1/- per gramme. The use of the vitamin in the beriberi area offers great possibilities for the prevention of infantile beriberi and the reduction of infantile mortality.

In last year's report reference was made to superficial keratitis due to riboflavin deficiency, which was described in India for the first time. Further work has been carried out on this disease, confirming previous findings. Some fifty cases were observed and treated in the Govt. Ophthalmic Hospital, Madras, and cases have also been observed and treated in Calcutta. Preliminary evidence has been obtained that certain types of angular conjunctivitis and blepharitis, without concurrent lesions of the cornea, may respond to riboflavin therapy. An investigation concerned with riboflavin deficiency was carried out in the Chingleput Leprosy Sanatorium with the cooperation of Dr. Cochrane. It was found that certain buccal and ophthalmic conditions ascribed to leprosy were in fact signs of riboflavin deficiency, and improved on treatment with riboflavin. There is little doubt that this member of the vitamin B_2 complex is of clinical and public health importance in India.

Preliminary observations have been made on partial optic atrophy, evidenced by pallor of the temporal half of the optic disc, which is common in patients attending the Ophthalmic Hospital, Madras. This condition, associated with partial failure of vision, is found in malnourished individuals showing evidence of deficiency disease. Treatment with vitamin A concentrates and yeast leads to slow improvement and it is probable that it can be added to the long list of diseases associated with malnutrition.

Indian children suffer much less from dental caries than English or American children. This observation has been further confirmed by a survey of dental caries in schools in Madras, in which some 2,000 children were examined. The incidence of caries in schools of different economic status was ascertained, children in poor, middle-class and well-to-do schools being investigated. The main difference between the diet of children of superior economic status and that of poor children is that the former includes foods other than rice—milk, pulses, vegetables and fruit—in greater quantities. It was, however, found that poor children did not suffer to any greater extent from caries than the children

of more prosperous parents, as is evidenced by the following figures referring to the incidence of caries in a poor and a well-to-do school for boys.

		Total number of teeth.	Percentage of teeth carious.	Extent of caries. 1	2	3	4	Average caries figures.
Deciduous	Poor ..	943	23.9	86	47	44	49	0.53
	Well-to-do ..	1,052	24.5	79	85	46	48	0.55
Permanent	Poor ..	7,379	6.2	330	67	33	29	0.092
	Well-to-do ..	13,174	6.0	522	132	79	62	0.097

Children with various vitamin deficiency diseases did not show more caries than other children. The relatively low caries rate in India, and the facts recorded above, are of importance in connection with the unsolved problem of the etiology of dental caries, and any satisfactory theory of origin must account for them. The clue to the problem may be found by the study of groups which are immune or relatively immune to the disease rather than by the study of populations showing a high incidence.

Investigations on yeast.

A small pilot plant for producing torula yeast has been in successful operation. The vitamin B_1, nicotinic acid and riboflavin content of various kinds of dried yeast was investigated. Dried torula yeast is as rich in vitamin B_1 and riboflavin as brewer's yeast and is suitable for medicinal use. Distillery yeast, on the other hand, is considerably lower in vitamin content. Typical values are as follows :

	Vitamin B_1 µg./g.	Riboflavin µg./g.	Nicotinic acid µg./g.
Dried brewer's yeast ..	20—40	45—60	400—500
„ torula yeast ..	20—40	60—80	150—350
„ distillery yeast ..	10—15	20—35	60—90

The protein content (N×6.25) of dried torula yeast is of the same order as that of dried brewer's yeast (35 to 50 per cent) while that of distillery yeast was in the neighbourhood of 30 per cent.

The value of dried torula yeast as a supplement to Indian and English diets was studied by growth experiments on rats. It was found that the addition of yeast to such diets increased the growth rate.

Training course in nutrition.

The usual course was given during April-June, the class including 16 members. The class was somewhat smaller than usual, owing to the fact that it coincided with the invasion scare. This was the sixth annual class.

EDUCATION AND PROPAGANDA.

The children's Health Bulletin " Food and Diet " was amended in accordance with the suggestions of last year's Nutrition Advisory Committee and was eventually published in October, 1942. Additions have been made to the museum, which now includes numerous pictures, charts and exhibits. Enquiries embracing many aspects of nutrition continue to be received from all over India.

6. Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

NARCOTICS AND VITAMIN C SYNTHESIS.

In continuation of the researches on the effect of certain narcotics on ascorbic acid metabolism, it has been observed that some of these narcotics produced increased elimination of ascorbic acid in the urine of rats. This increased elimination of ascorbic acid seemed to be due to increased synthesis of ascorbic acid in the body. These narcotics cannot apparently serve as direct precursors in the formation of ascorbic acid but they stimulate the formation of ascorbic acid by some unknown process. Narcotics are known to cause the following changes in the system, namely (1) lowering of the oxygen consumption, (2) disturbance in the normal carbohydrate metabolism and (3) inhibition of the activity of some particular dehydrogenases which are essential for normal metabolism. Experiments have been carried out to investigate the effect of certain physiological conditions on the process of ascorbic acid synthesis in order to discover the machanism of this synthesis. The conditions studied were those of (a) vitamin B_1-deficiency (b) anoxia due to lack of oxygen supply (c) anaesthesia (d) hypoglycemia produced by insulin injection and (e) inhibition of the process of respiration by poisoning the cytochrome system by cyanide and the succinodehydrogenase system by sodium malonate. Vitamin B_1 deficiency did not produce any increase in the excretion of ascorbic acid in the urine of the rat. The ascorbic acid content of the tissues like brain and liver was decreased while the ascorbic acid content of kidney tissue remained unaltered. Anoxia produced by the lack of oxygen supply did not appreciably increase the excretion of ascorbic acid in the urine of rats. The concentration in the kidney tissue was however increased. Ether anaesthesia showed increase in the concentration of ascorbic acid in all the tissues especially the kidney. Chloroform and coal gas seemed on the other hand to decrease the tissue content of ascorbic acid. Insulin injection made hardly any difference in the urinary excretion of ascorbic acid. Cyanide and malonate injections produced decrease in ascorbic acid excretion in the urine of rats.

VITAMIN C AND INCREASED METABOLISM.

During fever and infections of various kinds the excretion of free ascorbic acid in the urine has been found to be appreciably decreased and larger quantities of ascorbic acid are required to be administered in order to maintain the normal excretory level. The concentration of ascorbic acid in the organs like liver, kidney and adrenal has been found to be markedly diminished. This diminution in the concentration suggested the possibility of ascorbic acid being oxidized in the animal system due to increased metabolism. Experiments have been carried out to observe the effect of administration of desiccated thyroid, 2:4-

dinitrophenol and insulin on the urinary excretion of both free and combined ascorbic acid. The excretion of free ascorbic acid was found to decrease when guinea-pigs were fed with 0.065 g. of desiccated thyroid and also when fed with 2 mg. of 2:4-dinitrophenol. The excretion of combined ascorbic acid in both cases increased. Injection of 2 units of insulin produced very slight increase in both free and combined ascorbic acid. A decrease in the concentration of ascorbic acid in the tissues especially liver, kidney and adrenal was observed when desiccated thyroid, 2:4-dinitrophenol and insulin were administered to guinea-pigs. This investigation shows the necessity of ascorbic acid for the upkeep of the normal metabolism of the body. Increase in the metabolic acid rate utilises larger amounts of ascorbic acid.

Activity of blood serum esterase under different conditions of vitamin C nutrition.

The existence of a possible relationship between esterase activity and the concentration of ascorbic acid in the tissues like liver, kidney, intestine and blood of the guinea-pig, has been reported from time to time. No constant relationship between ascorbic acid content and esterase activity has so far been established. Raabe (*Biochem. Z. 299*, 141, (1938)) showed that only intravenous injection of ascorbic acid caused a temporary rise in the esterase activity in the liver. Recently King and coworkers (*J. Biol. Chem., 138*, 111, (1938)) have observed a progressive decrease in the esterase activity in the liver of guinea-pigs along with the progressive severity of scurvy. Experiments have been carried out to study the esterase activity of blood serum under different conditions of vitamin C intake, namely, during the normal, scorbutic and vitamin C- saturated conditions. It has been observed that in normal guinea-pigs, there were wide individual variations in the serum esterase activity depending on the degree of saturation of the body with ascorbic acid. In saturated conditions produced by feeding 50 mg. of ascorbic acid daily for 7 days to normal guinea-pigs, the serum esterase activity increased appreciably and more uniform results were obtained. When ascorbic acid was withdrawn from the food, the esterase activity decreased gradually but after 21 days of scurvy there was a sudden drop in the esterase activity. *In vitro* experiments, however, indicate that the esterase activity of scorbutic serum cannot be increased by simply adding ascorbic acid to the serum. Apparently, therefore, the influence of ascorbic acid on the esterase activity is more complex, and is under further study.

Intradermal test, urinary ascorbic acid, blood ascorbic acid and tissue ascorbic acid.

A detailed study has been made between urinary ascorbic acid, blood ascorbic acid and intradermal test time in both normal and scorbutic guinea-pigs. The experiments showed a want of correlation between the intradermal test time, the urinary excretion of ascorbic acid and the blood ascorbic acid level. Doubt is thereby cast on the conventional methods of assessing the vitamin C status of the body.

Investigations on the possible relationship between the intradermal test time and the ascorbic acid content of the liver have been carried out. This investigation shows that when the liver of an animal dosed with excess of vitamin C appears to reach a maximum value in ascorbic acid, the intradermal test time reaches a minimum value as indicated in the skin test. This investigation

indicates that the intradermal test affords a fair index for vitamin C saturation of the body. Experiments have also been carried out to establish the relation (if any) between the intradermal test time and the disappearance of combined ascorbic acid in the urine of guinea-pigs. The disappearance of combined ascorbic acid in the urine does not correspond with the minimum decolorisation test time in the skin-test.

COMBINED ASCORBIC ACID IN THE URINE. ITS STABILITY AND CONDITIONS OF SPLITTING.

Earlier, it has been observed that the combined ascorbic acid excreted in the normal urine was of a different type from that present in plant tissues. The combined ascorbic acid in the urine was found to be stable when preserved with 5% acetic, hydrochloric and sulphuric acids. Heating in an atmosphere of H_2S would split this combined ascorbic acid. It has been observed that the splitting of the combined ascorbic acid in urine can also be effected in an atmosphere of CO_2, if the *p*H is sufficiently lowered (to *p*H 1.6) and the solution heated on waterbath for one hour. With increasing *p*H the splitting diminishes more rapidly in an atmosphere of CO_2 than H_2S.

The fairly stable nature of combined ascorbic acid in the urine suggests the possibility of concentrating it by vacuum evaporation. This is being done and the concentrated material purified from salts by dialysis.

7. Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

VITAMIN A AND D CONTENTS OF FISH LIVER OILS.

The vitamin A and D contents of three different liver oils of Dhain (*Silonia silundia*), Vetki (*Lates calcifer*) and Sankhachur which yield fairly large quantities of oil have been investigated. Among the fish analysed, Dhain contains the highest vitamin D content, i.e. 6.67 international units per gm. of oil. Sankhachur and Vetki contain 5.0 and 2.86 units of vitamin D respectively. The vitamin A content of 563 international units per gm. of oil is the highest in Dhain. The liver oils of Vetki and Sankhachur contain 457 and 387 international units of vitamin A respectively per gm. of oil. It is clear that the vitamin A and D contents of the three different fish liver oils analysed are much less than that of the sample of cod liver oil (Squibb's) whose vitamin A content in international units has been found to be of the order of 1500, and whose vitamin D content is 300 units. It is evident that none of the samples of the fish analysed can be taken as good sources of vitamin A or D. Although Sankhachur was investigated particularly as a sea fish, it had really no advantage over the other fresh water fishes as a source of vitamin A or D.

EFFECT OF COOKING ON THE BIOLOGICAL VALUE OF FISH PROTEIN.

It has been reported earlier that in the process of cooking, the proteins very often undergo denaturation, hardening and drying, which to some extent affect their digestibility by proteases as studied under *in vitro* conditions. In continuation of the above work, the biological values of a few species of fish after different processes of cooking, have been investigated by the rat growth

method, which indicated that except in the case of Hilsa fish (*Clupea ilisa*) whose biological value of the protein was found to be greatest with fried fish and least with raw fish, the best results regarding the biological value of these fish proteins were obtained with underdone fish as compared with raw, fully cooked or fried fish. The change in the biological values of two more common varieties of fish proteins viz. Katla (*Catla catla*) and Vetki (*Lates calcifer*) after different processes of cooking has been studied. These findings are in agreement with the results reported earlier and with those of Clifford (*Biochem. J.*, 1930, *24*, 1729) who states that overcooked meat is very slowly digested as compared with underdone meat and the maximum rate of digestion is obtained with the latter.

Available iron in fish.

It has been reported earlier that about 30 to 40% of the total available iron in fish tissue is present in complex combination with nucleoprotein and copper, and this iron can be quantitatively estimated by Hill's dipyridyl method. So, Hill's conception regarding the inorganic nature of non-haemin iron in foodstuffs does not seem to be justified. The present investigations indicate further that the haemoglobin regenerative capacity of the iron-copper-nucleoprotein complex is much more pronounced than that of an equivalent quantity of pure iron salt when incorporated in the diet of anaemic animals. This result is contrary to the conclusion of Hill, which seems to be fairly generally accepted, and supports, in a way, the original idea of Bunge who stated that iron in firm combination with organic compounds as in haematogen could be used by animals for haemoglobin building. The iron in the iron-copper-nucleoprotein complex has been found to be present in the ferric state and it has also been found that only the ferric iron can combine with nucleoprotein *in vitro*. Investigations on the absorption mechanism of this iron from the study of isolated intestinal loops which retain their nerve and blood supply, indicate provisionally that ferric iron can be absorbed as such and no reduction is necessary prior to absorption from the alimentary tract.

Studies on the distribution of protein and nonprotein nitrogen and estimation of some individual aminoacid units indicate that the arginine content is the highest, viz., 17%. The histidine and lysine contents are 9.32 and 6.25% respectively. The proline content of the protein which has been found to be 4.3% might play some rôle in haemoglobin building. The purine nitrogen has been found to be 3.8% of the total nitrogen.

8. Studies on human nutrition with Indian diets under Dr. K. P. Basu at the Dacca University, Dacca.

Work on the metabolism of fats and of the trace element lead was started. Investigations on mineral metabolism with special reference to the mutual effect of minerals in metabolism and the rôle of vitamins were continued.

Fats.

The absorption of different vegetable oils and of vegetable and animal fats was studied by actual metabolic experiments with humans, The fats and oils

studied so far are mustard, cocoanut, sesame and groundnut oils, margarine, butter fat (cow and buffalo) and *vanaspati*. All these oils and fats showed high absorption, the percentage of absorption varying between 92 and 100. Administration of the different fats to young rats at 6, 9 and 12% level showed that maximum rate of growth was induced at 6% level of fat intake.

Mutual effect of minerals in metabolism.

Some interesting results were obtained about the mutual effect of minerals in metabolism. It was found that there was a mutual antagonistic effect of calcium, magnesium, iron and manganese in their metabolic behaviour. Removal of copper, iron and manganese caused by the administration of a high dose of calcium and magnesium salts took place mainly by way of the digestive tract. The addition of calcium lactate or gluconate to a low magnesium diet depleted the body of its magnesium, while high magnesium input possibly affected the process of calcification and depleted the bone of calcium. Iron and manganese salts added to a diet decreased the utilisation of calcium, magnesium and phosphorus. The present investigation seemed to offer an explanation for the development of rickets in animals caused by the addition of magnesium, beryllium, strontium, iron, aluminium, lead and other salts to the diet.

Rôle of vitamins in the metabolism of some essential elements.

Vitamin A, B-complex, C, D, the heat-stable fraction of the B-complex, and lactoflavin were found to possess beneficial effects on the utilisation of calcium, phosphorus, magnesium, and iron. Nicotinic acid on the other hand has got an adverse effect on the utilisation of the above elements. The anti-anaemic factor of the B-complex postulated by some workers seemed to be present among the heat stable factors.

Metabolism of lead.

Lead was determined by the colorimetric method, in which lead is precipitated as lead potassium chromate which after being dissolved in acid forms a red colour with sym-diphenylcarbazide. This method is simple and presents a distinct improvement in sensitivity and accuracy. Unlike the dithiozone method, preliminary separation of lead is not necessary and there is no interference by other metals.

Metabolism of lead was studied by feeding normal rice-fish diet to different human subjects. The following data were obtained :

Experimental subject.	Food lead (mg) per day.	Urinary lead (mg) per day.	Faecal lead (mg) per day.	Balance (mg)
R. B. A.	27.70	0.83	7.92	+ 18.95
G. C. D.	37.64	1.61	13.18	+ 22.85
,,	37.94	1.23	13.24	+ 23.47

The amount of lead present in food was thus quite considerable. Of the total amount of lead excreted in urine and faeces, about 10% came out through urine and 90% through faeces. Marked positive balance of lead was observed in the three cases examined. This may be due to two reasons : (1) it may be retained by the body much like other nutritional elements, or (2) it may be excreted by channels other than kidney and intestine, which alone have been considered here. The second possibility seems to be more likely since lead may be excreted from the body by other ways, e.g. as hair, which contains by far the largest amount of lead present in any human tissue.

9. Enquiry into calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

Previous work showed that the protein concentration remained within normal range, while the total calcium decreased in rickets. The value of $[Ca^{++}]$ calculated according to McLean and Hastings' equation also indicated a decrease in rachitic blood samples. Further observations on induced rickets in dogs have confirmed earlier findings on serum Ca^{++}. A study of the changes in the concentration of various constituents of blood serum in rickets revealed, that vitamin D influenced not so much the values of $[Ca^{++}]$ nor the concentration of phosphorus, as it did the state in which salts of calcium and phosphoric acid like $CaHPO_4$ and $Ca_3(PO_4)_2$ were deposited in the bone matrix. Thus the calculation of the respective ionic products viz., $[Ca^{++}] \times [HPO_4^{=}]$ and $[Ca^{++}]^3 \times [PO_4^{\equiv}]^2$ from the figures obtained for blood serum of both the experimental as well as the clinical type of rickets demonstrated, that these products decreased in a state of vitamin D deficiency.

The value of $p\,[Ca^{++}] \times [HPO_4^{=}]$ in equilibrated serum like salt solutions has been reported by other authors to be about 5.6. The value calculated from our observations lies in the neighbourhood of 5.7 and above for rachitic animals and below 5.7 for the protected or normal animals. Similar results are obtained in case of rachitic and normal children. The value of $p\,[Ca^{++}]^3 \times [PO_4^{\equiv}]^2$ in normal blood serum is 23.1. Our values for rachitic animals are higher and for protected animals lower than the above quoted figure. From these results it is clear that in a state of vitamin D deficiency the serum is undersaturated with respect to both the salts of calcium viz. $CaPHO_4$ and $Ca_3(PO_4)_2$. This may be one of the reasons for the lack of bone deposition in rickets and for the resorption of bone observed in vitamin D deficient animals. It is hoped that either of these products will give a better idea of the state of nutrition with regard to vitamin D than Ca × P product which is commonly in use.

Changes were observed in the concentration of albumin and globulin in the absence of any change in total proteins in rachitic and normal serum. This has led to the determination of the distribution of globulin fractions in the blood serum of experimental animals.

10. Enquiry into malnutrition of Bengali boys with reference to vitamins B and C deficiency under Professor N. M. Basu at the Presidency College, Calcutta.

The investigations carried out during the year are summarised below :

Vitamin C.

About fifty different kinds of herbs including plant leaves, flowers and their hips used in the preparation of Ayurvedic medicines were examined for their vitamin C amd carotene contents, precautions being taken to avoid loss in vitamin C due to drying. The vitamin C content of some of these was estimated after they were properly fried in oil. The results of analyses show (i) that *sprouting and tender neem leaves* are very rich in both vitamin C and carotene, viz., 500 mg of vitamin C per 100g and 75γ carotene per g, but these high values quickly go down on the maturing of these leaves ; (ii) that amongst the other herbs examined *Dhania, Vasak* and *Babla* leaves are also quite rich in both vitamin C (250 mg, 250 mg and 200 mg respectively per 100 g) and carotene (52γ, 45γ and 48γ respectively per g); (iii) that *Nishinda* leaves, *Marigold flowers and hips, Amrul, Shiuli* and *Bel* leaves are also fairly rich in vitamin C (100 mg or more per 100 g) ; (iv) *Pudina*, which is eaten raw, is richer in carotene (54γ per g) than vitamin C (16 mg per 100 g) and (v) that leaves of Dhania, bitter gourd (i.e. Uchche), Thalkuni, Kalmeg, Punarnaba and Marigold flowers do not lose their store of vitamin C when fried in oil, whereas leaves of Nishinda, Ayapan (i.e. Bisalya-karani) and Shiuli gain in vitamin C to nearly double their original content on frying in oil.

Vitamin B_1.

(a) *Experimental studies on the influence of vitamin B_1 on the inflow of phosphates in the intestinal canal and the absorption of glucose therefrom :—* Rabbits starved previously for 24 hours were anaesthetised with urethane. After laparotomy was done, the small intestine was washed with warm Tyrode solution (without phosphate and glucose) injected just below the pyloro-duodenal junction and then moved down slowly by mild pressure. It was then ligated so that 2 loops, each of 30 cm. in length were obtained from jejunum. In one of these warm hypertonic solution of glucose and in the other the same solution plus 10γ of thiamin were injected. In some animals warm Tyrode solution (without glucose and phosphate) was injected in one loop and the same solution with 10γ of thiamin in the other loop. The amount of glucose and phosphates in the former series of experiments and of phosphates alone in the latter series was ascertained in an aliquot portion of the contents of the loops 20 minutes after the injection, and in the whole fluid in each loop 1 hour after the injection. The animals, particularly their abdomen, were artificially kept warm during the period of the experiment.

The results of estimation of phosphates and sugar in these loops are as follows :—

INFLOW OF PHOSPHATES IN μg.

In loops containing phosphate-free Tyrode		In loops containing Phosphate-free Tyrode plus vitamin B_1 (from 5 to 40 μg)		In loops containing isotonic glucose solution.	In loops containing isotonic glucose solution.	In loops containing isotonic glucose solution plus vit. B_1 (10 μg)	In loops containing isotonic glucose solution plus 10 μg vit. B_1
After 20 mts– in 1 cc of the intestinal content	After 1 hr– in the remaining intenstinal content	After 20 mts.	After 1 hr.	After 20 mts.	After 1 hr.	After 20 mts.	After 1 hr.
49 μg (average of 2 experiments)	84.2 μg (average of 2 experiments)	49.3 μg (average of 7 experiments)	222.8 μg (average of 7 experiments)	156 μg (average of 5 experiments)	241.4 μg (average of 5 experiments)	163.4 μg (average of 5 experiments)	289.4 μg (average of 5 experiments)

ABSORPTION OF SUGAR.

From loops containing isotonic glucose sol		From loops containing isotonic glucose sol plus 10 μg vit B_1	
After 20 mts.	After 1 hr.	After 20 mts.	After 1 hr.
glucose content in 1 cc of the fluid in mg	glucose absorbed in mg (by deducting from the total amount the sum of the amounts present in the loop after 1 hr. + that present in 1 cc. of the fluid taken after 20 mts.)	glucose content in 1 cc. of the fluid in mg	glucose absorbed in mg (obtained in the same way as in col. 2.)
58.5 mg (average of 5 experiments)	164.3 mg (average of 5 experiments)	51.2 mg (average of 5 (experiments)	196.4 mg (average of 5 experiments)

These results show clearly that the presence of vitamin B_1 in the intestine definitely increases the inflow of phosphates in, and the absorption of sugar from, the intestinal loops, this inflow being further increased by the presence of sugar. The presence of sugar alone causes also a marked increase in the inflow of phosphates. It is to be noted that in one experiment only with an adult rabbit the phosphate inflow after an hour in the loop containing glucose and vitamin B_1 was somewhat less than in the loop containing glucose alone (viz. 360 μg vs. 400 μg) and the absorption of glucose from the former was also less than from the latter (viz. 140 mg vs. 155 mg). It appears from this that the increased absorption of sugar from a loop containing vitamin B_1 is chiefly due to the effect of the latter on the inflow of phosphates. To ascertain how far this apparent conclusion was true or not, experiments were made for finding out the effects of the presence of different amounts of vitamin B_1 in the intestinal loops on the phosphate inflow and the absorption of sugar. It was found that the absorption of sugar is not exactly correlated with the amount of inflow of phosphates and is not also increased by increasing the amount of vitamin B_1 (from 10 μg to 50 μg).

(b) *The influence of Vitamin B_1 on the basal metabolic rate (B.M.R.) and carbohydrate utilisation of human beings.*—The B. M. R. of students was ascertained indirectly by a modified Krogh's closed-circuit apparatus by determining the oxygen consumption. They were then each given 50.0g of dextrose and the oxygen consumption was noted for 6 minutes each time after every half hour till 2 hours after the ingestion of glucose. Simultaneously the sugar content of blood was examined after every half hour.

It was found that, after the ingestion of glucose, oxygen consumption increased appreciably but fell down after one and a half hours to a level lower

than the original level. Thus oxygen consumption after the ingestion of glucose takes a course parallel to that shown by the sugar content of blood.

These students were then given a daily dose of 3 mgs. of vitamin B_1 for a week in order that their B. M. R. might be again examined and the influence of glucose ingestion on their oxygen consumption and the glucose content of their blood might be recorded. Unfortunately, on account of disturbances in Calcutta, this work could not be completed.

(c) *Influence of vitamin B_1 on the chronaxie of animals and humans* :— The chronaxie values of 40 individuals were recorded and were found to vary between 0.12 to 1.0 σ (σ = micro-second). These values were usually higher immediately after meals and sleep and were minimal in the morning before meals. The chronaxie values of vitamin B_1-deficient rats were significantly higher than those of normal animals.

(d) *The influence of vitamin B_1 on detoxication by liver*:—The detoxication of chloral hydrate by conjugation with glycuronic acid formed in liver was found to be less efficient in vitamin B_1-deficient rats, only when their liver was partially damaged by CCl_4.

11. Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lt. Col. E. W. O'G Kirwan, Professor of Ophthalmic Surgery, Medical College, Calcutta.

The work is being carried out at the Eye Infirmary, Medical College Hospitals and in the districts of Bengal in collaboration with the Association for the Prevention of Blindness in Bengal. During the early part of the year, up to March, investigations were carried out to find the rôle of vitamin A in the prevention of blindness and eye diseases in Bengal. From April investigations are being carried out to find the rôle of vitamin B_2 (robiflavin) in the etiology of eye diseases and the incidence of this deficiency in Bengal.

Work on vitamin A.

At the Eye Infirmary of the Medical College Hospitals, Calcutta, 19,509 patients were examined, of which 154 cases had symptoms or signs of vitamin A deficiency. The distribution of the cases was as follows :—

Nightblindness	..	58
Keratomalacia	..	39
Pigmentation, xerosis of the conjunctiva or Bitot's spots	..	57

Field work was carried out in the district of Bankura. Investigations were carried out in jails, schools, and amongst the patients attending the outdoor department of the travelling eye dispensary working in Bankura district. In all, 1,682 cases were examined for vitamin A deficiency. One hundred and sixty-four cases showed pigmentation and xerosis of the conjunctiva. Biophotometric examination was carried out on a group of 192 cases, who were given the same diet and who lived under the same conditions. The results of the investigation were as follows :—

(1) Fifty-eight cases showed pigmentation or xerosis of the conjunctiva, of these twenty-four cases, i.e., 41.3% were found to be deficient in vitamin A.

(2) One hundred and thirtyfour cases did not show any pigmentation or xerosis of the conjunctiva ; 60 of them, i.e. 44.7% were found to be deficient in vitamin A content in their system. The diet was found to be deficient in vitamin A. The cases on the whole were fairly intelligent and the results of biophotometric examination were fairly reliable.

WORK ON VITAMIN B_2 (RIBOFLAVIN).

From the month of April, investigations are being carried on vitamin B_2 (riboflavin) as a cause of ocular disease. At the Eye Infirmary, Medical College Hospitals, Calcutta, out of 15,203 cases, 18 only showed one or more of the general signs of this deficiency and of these 8 cases only showed ocular manifestations. The distribution was as follows :—

Keratitis	.. 3
Angular conjunctivitis and blepharitis	.. 4
Corneal ulcer	.. 1

Investigations were carried out in orphanages, jails and amongst the workers in a jute mill. One thousand five hundred and thirtyone cases were examined and the result was as follows :—

Angular stomatitis	.. 86
Angular stomatitis with lesions of tongue	.. 62

Ocular signs were not found in any of these cases. Field work was carried out in Kishoreganj in the district of Mymensingh. Investigations were carried out in the schools, villages, market places and on the patients attending the outdoor of the travelling eye dispensary working in this district. One thousand four hundred and ninetyone cases were examined and the result of examination was as follows :—

Angular stomatitis	.. 34
Angular stomatitis with lesions of tongue	.. 23

None of these showed any ocular signs. It would appear that diseases of the eye due to riboflavin deficiency are not common in Bengal. The investigation is being continued.

12. Studies on the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

The object of this enquiry was to determine the conditions under which carotene is utilised in the body as vitamin A. The main difficulty experienced in a work of this nature is that of estimating very low concentrations of vitamin A particularly in association with sterols largely present with fats. The spectrophotometric method largely depended upon, revealed its unreliability in

low concentration assays of vitamin A. Absorption of certain regions of ultra-violet light near about that specific to vitamin A can also occur, with certain other as yet undefined substances. It is, however, very difficult to remove the impurities completely, and to obtain the true value of vitamin A absorption, one has to resort to indirect methods of destroying the active principle. So far these methods consisted in destroying vitamin A by ultraviolet light and comparing the extinction coefficient before and after such destruction. However, certain tests with known amounts of added vitamin A proved, that this method is not satisfactory as the potency estimated by this method was always low. It was therefore considered, that methods of chemical oxidation of vitamin A may prove more helpful.

Several experiments were performed utilising varying amounts of sodium peroxide as an oxidising agent during the process of saponification and concentration of vitamin A. It was found that too much heat was generated by adding sodium peroxide and therefore this method too was found unsatisfactory for routine work. However, by the use of hydrogen peroxide it was possible to obtain consistent results with known amounts of added vitamin A.

A few grammes of margarine was saponified and the saponified solution was divided into two equal portions. One portion was extracted with ether in the usual way and the other portion was shaken with a few c.c. of hydrogen peroxide for oxidising vitamin A and then extracted with ether. In order to stimulate the oxidising action, a few c.c. of potassium permanganate solution was added after the addition of hydrogen peroxide, and the whole mass was shaken for a few minutes. Care was taken not to add too much of the oxidising reagents lest this should oxidise some of the coloured pigments. In this process, the double saponification needed with sodium peroxide is avoided and the materials causing irrelevant absorption are present in equal amounts in the two cells of the spectrophotometer. The potency estimated by this method approaches the true value, and the results of these experiments as well as a standard method developed are described in a paper that is being written up.

The different isomers of carotene having different biological values are separated by the chromatographic method on a Tswett column packed with various adsorbents. Due to the present war crisis the special type of adsorbents mentioned in literatures are not available and therefore attempts have been made to improve the quality of adsorption by utilising those adsorbents available in the market. It was found that the rate of percolation of a solution of carotene in petroleum ether was very slow when magnesium oxide powder only was packed inside a Tswett tube. In this connection use was made of powdered silica gel which was a bye product of another work. Better adsorption and separation has been noticed and it is proposed to extend these experiments as it holds out promise of value. This knowledge of the chromatographic separation of the different isomers of carotene and their subsequent spectrophotometric estimation would be of great help in our future studies.

13. Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay.

The object of the enquiry is to see how far the various tests of physical fitness used in England and America are applicable to Indians, and to work

out the standards for Indians in these respects. No such data have so far been worked out in this country.

During the year observations on the following tests were extended to more than 600 individuals mostly in the age group 18 to 29 years.

The following is a brief summary of the observations.

Circulatory tests.

These are mainly in use in America for recruitment to the A. A. S. and for determining staleness and fatigue among members on active service.

(1) *The Schneider test of cardiovascular rating*—In this test the number of points scored according to a scheme devised by Schneider based on six factors, viz., (i) recumbent pulse rate, (ii) standing pulse rate, (iii) difference between the two, (iv) difference between the standing rate and the pulse rate after a standard exercise, (v) time of return of the post exercise to the standing pulse rate, and (vi) difference between the recumbent and the standing systolic blood pressure, serve to grade the physical fitness of an individual. A score below 8 is considered as suspicious of an unfit condition for an American.

It was seen here that in 86 per cent of 418 normal males, leading the usual life with none engaged in any special athletic exercise, the score was 8 or above. Amongst athletes the percentage was 97 in a group of 34. When the same age group was considered however the percentage was 100 in a group of 19. In diseased persons with some under treatment the percentage was only 59. Among females 68% of the normal and 37% of the diseased group scored at least 8 points.

The 'reliability coefficient' of the test was determined by repeating it on 27 persons, male and female, under practically identical conditions but on different days, and then correlating the results statistically. It was found to be 0.81, a significant figure, and the test may therefore be said to be sufficiently accurate to measure what it purports to measure.

A statistical study was undertaken. The individuals were divided into three groups comprising (1) those taking no regular exercise of any kind, (2) those taking moderate exercise such as walking or playing a game regularly, and (3) those taking controlled athletic exercises in recognised gymnasia. By using the method of significance of difference between means, it was seen, that the test could well separate out the athletic group from the non-athletic, and in the latter, the 'no exercise' group from the 'moderate exercise' one both in the males and females separately. If we therefore assume, that exercise improves the physical fitness of an individual, this test may be said to indicate the improvement objectively and thus may be used in grading improvement and fitness. In a group of 35 athletes the mean score was 13.76 and while 54 per cent of them could secure this score, only 22 and 36 per cent respectively of the 'no exercise' and the 'moderate exercise' groups could score that much among the males.

Using the same method a significant difference was found between the mean scores of the corresponding 'no exercise' and 'moderate exercise' groups of the males and females thus separating out the sex influence on the score.

It was also noted that the time of day and the diet probably do not influence the score of a person.

The influence of the socio-economic habits on the score was studied by comparing the mean scores of the different communities, viz., Gujerathis, Deccanis,

other Hindus, Parsis, Muslims, and Christians, both in the 'no exercise' and in the 'moderate exercise' groups. No statistically significant difference was found between the scores of the different communities.

The correlation of the test with age and body measurements is being determined as also its 'validity' by correlating the results with those obtained by independent clinical grading.

The mean Schneider scores obtained in some of the groups are given in Table I.

Table I.

	'No exercise' group		'Moderate exercise' group		'Athletic' group	
	Males.	Females.	Males.	Females.	Males.	Females.
Schneider score.						
Mean with P.E.	11.17±0.15	9.53±0.30	12.75±0.16	11.08±0.46	13.76±0.29	..
Standard deviation	3.41	3.42	2.79	2.98	2.51	..
Number of individuals in group.	249	59	134	19	35	..

(2) *The Crampton 'blood ptosis' test.*—Crampton prepared a percentage scale of fitness by adjusting the postural changes in pulse rate in terms of corresponding blood pressure variations. For American males he takes 50 as the minimum score for fitness.

In our series of observations 96 per cent of the ordinary normal males, all the athletes, and 86 per cent of the diseased males secured this score. Normal and diseased females with similar scoring were 95 and 75 per cent respectively. The difference between the mean scores of the male 'no exercise', 'moderate exercise' and 'athletic' groups was in each case found to be statistically significant though not to the same extent as in the case of the Schneider Test.

(3) *The McCloy Test.*—In this the score is calculated from standing pulse rate and standing diastolic blood pressure by using a formula. The minimum index of fitness for males in America is given as zero.

In the cases observed here 59% of the normal males, 73% of the athletes and 52% of the diseased males secured this minimum. Among the females the percentages securing it in the normal and diseased groups were 31 and 25 respectively.

Respiratory tests.

These tests are mostly in use in England for recruitment to the R. A. F.

(1) *The breathholding test*—The minimum time of holding the breath for R. A. F. recruits in England is given as 45 seconds. Here 70% of the normal, 72% of the athletes and only 11% of the diseased could hold the breath as long. Among normal and diseased females the percentage was 26 and 0 respectively.

(2) *The expiratory force test*—Raising a column of mercury to 80 mm. is the minimum allowed for recruitment to the R.A.F. Here 66% of the normal, 74% of the athletes (79% of those in the same age group), and only 16% of the diseased could raise the mercury to this height. Among normal and diseased females the percentage was 14 and 0 respectively.

(3) *The persistence or fatigue test*—In this the mercury column is raised to half the height of the expiratory force test and held there for as long as possible. The average sustaining time for the males in England is given as 40 seconds. The minimum figure is probably much lower than this. Here only 36 per cent of the normal and 1% of the diseased could sustain for as long a time at least. Strangely, only 24% of the athletes could hold it for 40 seconds or more. When the same age group was considered however the percentage was 32. Amongst normal females the percentage was only 7 while that in the diseased females was zero.

(4) *Vital capacity*—3400 c.c. is given as the minimum vital capacity for R. A. F. recruits in England. Here only 38% of the normal, 35% of the athletes (42% of those in the same age group) and only 8% of the diseased could reach this figure among males while only one female could blow up to that extent.

(5) *Flack-Woolham index of fitness*—This is calculated from the above tests by using a formula given by the authors. The results here are rather very low.

Table II summarises the various percentage figures for the different tests and groups given above.

Table II.

Percentage of persons in the various groups securing the minimum score laid down for each test for male recruits to the A. A. S. and the R. A. F.

Test and minimum score laid down.	Groups.	(The figures in brackets indicate No. of individuals in the group).			
	'Non-athletic' males. (418)	'Athletic' males (34)	'Diseased' males (73)	'Normal' females (83)	'Diseased' females (16)
Schneider score (minimum 8 points)	86%	97% (100% in same age group)	59%	68%	37%
Crampton score (minimum 50)	96%	100%	86%	95%	75%
McCloy score (minimum 0)	59%	73%	52%	31%	25%
Breathholding (minimum 45 seconds)	70%	72%	11%	26%	0
Expiratory force (minimum 80 mm. Hg.)	66%	74% (79% in same age group)	16%	14%	0
Persistence Test (average 40 seconds, minimum ?)	36%	24% (32% in same age group)	1%	7%	0
Vital capacity (minimum 3400 cc.)	38%	35% (42% in same age group)	8%	One individual	0

GENERAL BODY MEASUREMENTS.

In every case the following measurements were recorded viz., height, (standing and sitting), weight, surface area, chest (resting, inflated, deflated, and mean), trunk area (chest × sitting height), abdomen, and nutritional indices (v. Pirquet's, Rohrer's, and Davenport's).

Statistical studies are being undertaken on all the tests and measurements on the lines indicated already under the Schneider test.

14. Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Dutta at the Grant Medical College, Bombay.

"The extraordinary slowness with which practice follows knowledge in medicine is nowhere more evident than in the study of the body proteins." Our knowledge of the functions of the plasma proteins in maintaining fluids in the vessels was given a scientific basis by Starling in 1896. It is after 100 years that we have at least reached an era where the body proteins are receiving some recognition. In the present investigation attempts are being made to verify and pass into practice as rapidly as possible the results of various uncoordinated individual researches. Determinations of plasma proteins have been carried out in more than 400 patients suffering from different diseases. The results obtained has been classified under two heads with a view to dertermining

(1) the relationship of plasma proteins to the occurrence of oedema of various types, and

(2) the alteration in the value of different protein fractions in different types of infection.

Plasma proteins in relation to oedema.

Any type of oedema occurs as a result of complex mechanism dependent primarily on the following factors, which may occur alone or in combination of two or more : (i) a decreased osmotic pressure of the plasma proteins, (ii) increased capillary permeability, (iii) increased capillary blood pressure and (iv) a decreased lymphatic drainage. The first clinical problem to determine the cause of oedema is undoubtedly hypoproteinemia.

(a) *Nutritional oedema.*—Hypoproteinemia is a constant finding in all cases of nutrition oedema, caused either by a lack of dietary protein or by imperfect utilization of the ingested protein. The plasma proteins, chiefly albumin determines the colloid osmotic pressure and play an important part in the distribution of extracellular fluid. Oedema is likely to occur when the colloid osmotic pressure is less than 15 mm. of Hg. (or 20 cm. of H_2O). Observations on 20 cases of nutritional oedema showed that the total protein was always less than 5 per cent. and albumin less than 2.5 per cent. The colloid osmotic pressure gave values, in most of the cases, well below the level at which oedema is expected to occur. (table I.)

(b) *Oedema assocciated with anaemia.*—The plasma proteins are usually low in all cases of anaemia. Anaemia is frequently associated with oedema. When the two conditions exists together, the oedema appears at a higher protein level than when hypoproteinemia is present alone as in purely nutritional oedema. The oedema level of colloid osmotic pressure in anaemia was found to be 21.84, standard deviation ± 2.84. All the 25 cases of anaemia without oedema gave an osmotic pressure above 24.4 mm. of Hg. The average total protein was 6.17 ± 1 per cent., with an albumin level of 3.86 ± 0.67 which was higher when compared with the protein values of those with oedema.

(c) *Cardiac oedema.*—Thompson in 1934 suggested that plasma protein deficiency plays an important part in the aetiology of cardiac oedema. Observations on patients of heart disease with and without oedema indicate that the primary change is a decrease in albumin and a rise in the globulin fraction of plasma protein. The extent of the rise in globulin determines whether the

total protein is normal or below normal. There was no significant difference between the two groups in the value of colloid osmotic pressure, which was found to be 24.4±3.6 mm. of Hg. in those with oedema and 24.67±3.8 mm. of Hg. in those without oedema. The albumin value in both groups was found to be 3.6 per cent, and was mainly responsible for the colloid osmotic pressure.

Deficiency of proteins does not appear to be a contributory factor in the causation of cardiac oedema, which is however likely to occur whenever the colloid osmotic pressure is less than 24.6 mm. of Hg.

(d) *Renal Oedema.*—An abnormally low albumin and an increased globulin content of the plasma is characteristic of the oedema of all cases of sub-acute nephritis. In all the cases (20) of oedema of nephritis the globulin made up 70 per cent of the total protein. The total protein was less than 5 per cent with an albumin content of less than 1.5 per cent, in every case. The colloid osmotic pressure was only 12.8 mm. of Hg.

Hypoproteinemia of nephritic oedema is due to loss of body protein. Protein loss in urine cannot account for such a subnormal protein concentration in the blood plasma. Defective protein formation may play a part in these patients in whom hypo-proteinemia is present.

In acute cases of nephritis, the albumin was higher than those of sub-acute type and was found to vary from 3 to 4 per cent ; the globulin was also higher. The mean colloid osmotic pressure was about 27 mm. of Hg., which is well above the oedema level.

(e) *Oedema of toxic pregnancy*—Oedema seen in toxic pregnancy is due to hypoproteinemia. Observations on 6 cases only, showed that oedema appears at a higher protein level with an average osmotic pressure of about 18 mm. of Hg., which is higher than those in the case of nutritional oedema.

Table I.

Summary of the protein changes in different types of oedema.

Type of oedema	Number of cases	Total Protein percentage	Albumin percentage	Globulin percentage	Osmotic pressure in mm. of Hg.
Nutritional oedema	20	4.36±0.4	2.06±0.7	2.22	15.1±3
Anaemia and oedema	14	5.71±0.63	3.33±0.35	2.25	21.8±2.8
Anaemia and no oedema	25	6.17±1	3.86±0.67	2.45	24.4±3.6
Cardiac oedema	10	6.60±0.7	3.60±0.8	2.9	24.4±3.6
„ no „	12	7.21±0.74	3.63±0.7	3.53	24.67±3.8
Nephritis oedema	20	4.81	1.49	3.17	12.94
„ no „	10	7.70	3.83	3.95	26.9
Eclampsia	8	5.64	2.36	3.37	18.02

THE PLASMA PROTEIN CHANGES IN VARIOUS INFECTIONS.

A study of the protein changes in infections may serve as a useful indication either in detecting or in finding out the severity of infection. The protein changes in infections consist in the main of a reduction of albumin and increase in globulin fraction of the blood plasma. The determination of total protein alone leave much to be desired and may lead to erroneous conclusions. The frequent practice of reporting plasma proteins in terms of total protein and albumin/globulin ratio, appear to have little significance.

The albumin, globulin (euglobulin and pseudoglobulin) and fibrinogen with the varied functions attributed to them should be treated as separate entities. Such a study will furnish the necessary foundation for the interpretation of results in diseases.

NORMAL STANDARD FOR THE SEPARATE PROTEIN FRACTIONS.

A normal standard has been worked out for healthy adults, taking care to rule out those who might present variation due to infection or recent immunization. Instead of albumin (A/G) ratio, the globulin per cent of the total protein has been included in the result, since it gives better indication as to the extent of infection. Any value above 39 per cent should be looked upon with suspicion.

Table II

Normal standard for separate protein fractions.

Average of	Total protein percentage	Albumin percentage	Total globulin percentage	Globulin percentage of total protein	Eu globulin percentage	Pseudo globulin percentage
20 males	7.56±0.25	5.05±0.21	2.44	33±2	0.55	1.67
4 females	7.62	4.88	2.81	36.9	0.59	1.73

Fibrinogen varied from 0.19 to 0.49%

Kala-azar—Most marked alteration of plasma protein was observed in a typical case of kala-azar admitted to the hospital with a total protein of 11.3% albumin 2.2% ; and globulin 9.06%. The globulin content is one of the highest figures recorded. The progressive rise of albumin following clinical improvement ; from a value of 2.27 to 4.44% during 58 days tends to verify that infection interferes with the production of albumin but regeneration occurs rapidly after termination of infection, regardless of high globulin which persists for some time. The decrease of globulin from 9.06 to 5.31 was due mostly to the reduction of euglobulin fraction (from 4.02 to 1.28%). Fibrinogen value remained practically constant throughout.

Malaria.—The changes in protein in malaria are similar to kala-azar, but the increase in the different globulin fractions is less marked. Increase in fibrinogen was observed in patients during incubation periods when no fever was present.

Liver has been considered as the main source of albumin and much of globulin. It is the sole source of fibrinogen. A decrease in albumin, increase in globulin and an increased fibrinogen cannot be explained unless one predicts as Loeb (1941) has, that albumin, globulin and fibrinogen are formed by different cells of the liver. A detailed investigation of different cases of malaria with treatment may solve many problems as to the site of formation of the body proteins.

Tuberculosis—The determination of total protein and the albumin/globulin ratio gave little indication as to the severity of the diseases in those cases which were studied in earlier investigations. Subsequent studies include determinations of different globulin fractions and also fibrinogen, in addition to albumin and total globulin. The average protein changes in 20 cases of tuberculosis is as follows :

Total protein	Albumin	Globulin	Globulin percentage of protein	pseudoglobulin
		Percentage		
7.15	3.24	4.02	56.2	2.37

Euglobulin and fibrinogen showed wide variations. Results of the individual cases showed that in those where the globulin percentage of the total protein is less than 50, the euglobulin and the fibrinogen was only slightly above the normal. In those patients where the globulin percentage of the total protein was above 56, the euglobulin and the fibrinogen was found to be higher. The pseudoglobulin was proportionately reduced. The decrease in albumin is probably related to the extent of wasting of the tissue protein. The exact significance of these findings can only be known by repeated observations on the same patients. The work is in progress.

Nephritis.—An increased globulin due to infection was observed in all the 16 cases of nephritis in which fractionation of the proteins was carried out. The increase in globulin was found to be due to the euglobulin and the fibrinogen fractions ; the pseudoglobulin fraction remained practically constant. In those cases of nephritis which are termed as the degenerative type in which the cholesterol values are abnormally higher, the pseudoglobulin was considerably reduced.

Cirrhosis of the Liver.—Only 5 cases of cirrhosis of the liver have been studied so far. The results showed a decrease in albumin, and increased globulin. The fibrinogen was either normal or slightly raised. The average value of the total protein was 6.52% with an albumin content of 2.69%. The ascitic fluid was found to contain both albumin and the globulin fractions. The total protein of the ascitic fluid varied from 1.25 to 1.68%. Further work is in progress.

Further studies include cardiac diseases, syphilis and tetanus.

Protein changes in normal and toxic pregnancies.

A detailed investigation is being carried out on the protein changes in blood plasma in a number of cases of normal and toxic pregnancies. Attempts are being made to study the blood protein in pregnant women from the

ante-natal stages and to find out the extent to which plasma proteins play their part in the development of eclampsia of pregnancy.

Investigations of 8 cases of eclampsia showed that the total protein was usually very low due to a decrease in albumin. The average of 8 cases gave a value of total protein of 5.64% with an albumin content of 2.36%; the globulin was 3.37% and the colloid osmotic pressure was 18.02mm. of Hg.

Alteration of plasma proteins occurs in different diseases. The utilization of these changes either for the diagnosis or for the prognosis demands a series of investigation on each disease and also a repeated investigation on the same case during treatment. What is more needed is a close co-relation of the laboratory finding with clinical observations. If this could be done, the determination of plasma proteins will play an important part in clinical medicine.

15. Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

Thirtyseven samples of human blood serum were analysed for the determination of the different lipoid constituents. These samples were obtained from healthy adults between 20 and 40 years of age. Out of these 37 subjects, 22 belonged to the middle class with a liberal fat intake and the remainder were from a group of poor class people whose fat intake was extremely low. The fat intake has been estimated from diet surveys and the figures have been found to be between 56 and 126 gms. in the case of the former group whereas in the latter group the fat intake was very much lower.

The following determinations were made on all samples of blood serum. (1) total lipoids i.e. total fatty acids + cholesterol (2) cholesterol (3) iodine value of the total fatty acids and (4) lipoid phosphorus.

Bloor's method (J. Biol. Chem., **77**, 53, 1928) for (1) and (2), Yasuda's method (J. Biol. Chem. **94**, 401, 1931) for (3) and the method described by Peters and Van Slyke (Quantitative Clinical Chemistry, Methods. P. 884, Edn. 1932) for (4) were adopted.

The results obtained so far are given in the table below. For the present, values for both the groups have been included in the table.

Table.

Concentration of serum lipoids in normal individuals.

	Total lipoids mg. p.c.	Total fatty acids. mg. p.c.	Cholesterol mg. p.c.	Lipoid p. mg. p.c.	Iodine value of fatty acids.
Maximum	692.4	569.6	203.8	15.88	171.4
Minimum	503.2	275.8	122.8	8.72	63.6
Average	591.3	427.0	164.3	11.42	125.2

In order to arrive at a definite conclusion as regards the influence of fat intake on the different constituents of the serum lipoids, at least 100 samples of serum in each group will have to be analysed. No comment can therefore be made on the results obtained so far.

16. Enquiry on the rôle of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

The enquiry started on the 6th May, 1942 and the following results were obtained.

MANGANESE CONTENTS OF THE SMALL INTESTINES AND LIVER OF DIFFERENT SPECIES OF ANIMALS.

It has been found that the manganese contents of the small intestines and livers of man and the guinea-pig are definitely lower than those of other species able to synthesise their requirement of ascorbic acid (table 1).

Table 1.

	Man.	Guineapig.	Rat.	Rabbit.	Goat.	Fowl.	Pigeon.	Remarks.
Small intestines	.47	.71	..	1.59	1.05	1.29	1.17	mg/100g dry.
	..	.14	..	.18	.17	.26	..	mg/100g moist.
Liver	.50	.62	..	1.01	1.19	1.05	1.29	mg/100g dry.
	..	.17	.27	.26	.35	.25	..	mg/100g moist.

SYNTHESIS OF ASCORBIC ACID BY GUINEAPIGS.

Guineapigs kept on a scorbutic diet and injected with manganese chloride solutions (as $MnCl_2, 4H_2O$) with or without glucose have been found to synthesise ascorbic acid in the jejunum (table 2).

Table 2.

Ascorbic acid content in jejunum and liver of guineapigs on scorbutic diet for 2 weeks. mg/lg.

	Jejunum	Liver		
Gr. I—	.05	.069	Gr. I—	Controls, Basal diet only.
Gr. II—	.04	.067	Gr. II—	Basal diet + glucose injection.
Gr. III—	.11	.080	Gr. III—	Basal diet + Mn injection.
Gr. IV—	.10	.097	Gr. IV—	Basal diet + Mn + glucose injection.

Taking the mean of gr. I + gr. II as the mean ascorbic acid contents of guineapigs tissues on scorbutic diet for 2 weeks it is found that gr. III (Mn) synthesises, 0.055 mg. and gr. IV (Mn + glucose) synthesises .065 mg. ascorbic acid per gram of jejunum.

Guineapigs on the basal diet for one day only and injected with manganese were found to synthesise ascorbic acid in the jejunum to a greater extent (table 3).

Table 3.

Ascorbic acid contents of guineapig tissues and the amounts synthesised by them.

1	2 Gr. I	3 Gr. II	4 Gr. III	5 Gr. IV	6 Mean of 2+3	7 4—6	8 5—6
Jejunum	.20	.15	.24	.25	.175	.065	.075
Liver	.116	.09	.12	.125	.103	.017	.022

The groups are the same as in the previous experiments. Guineapigs receiving only Mn synthesise 0.065 mg (col. 7) and those receiving Mn and glucose synthesise 0.075 mg (col. 8) ascorbic acid per gram of tissue. The synthesis in the liver is not prominent in both sets of experiments.

SYNTHESIS OF ASCORBIC ACID BY RABBITS.

Young rabbits weaned recently were kept on milk diet for 1 week. One group was supplemented with manganese, the other group was kept as control. Each group was divided into two sub-groups, one receiving vitamin B complex and the other not receiving it. For the second week they were kept on a synthetic diet. After 2 weeks, the jejunum of the 2 groups receiving manganese showed a much higher ascorbic acid content than the groups not receiving it, showing synthesis by the groups receiving manganese. Vitamin B complex has no influence upon the synthesis of ascorbic acid by rabbits (tables 4 and 5).

Table 4.

(mg ascorbic acid/lg tissue)

	Gr. I	Gr. II	Synthesis Gr. II
Jejunum	.22	.29	.07
Liver	.11	.13	.02

Table 5.

(mg ascorbic acid/lg tissue)

	Gr. I	Gr. II	Gr. III	Gr. IV	Synthesis II	Synthesis III	Synthesis IV
Jejunum	.22	.41	.22	.40	.19	nil.	.18
Liver	.12	.16	.13	.165	.04	.01	.045

I—Basal diet only; II—Basal diet + Mn only; III—Basal diet + B only; IV—Basal diet + Mn + B.

Rabbit liver incubated in Ringer-Lock solution synthesises 0.07 mg of ascorbic acid per gram in presence of .0005% added manganese.

POSSIBLE SYNTHESIS OF ASCORBIC ACID IN MAN.

A young man (N.C.B.) was found to excrete normally in the urine 14 mg of ascorbic acid daily. Blood ascorbic acid was 0.8 mg per cent. The average daily intake of available ascorbic acid was 25—30 mg. The same person was kept on ascorbic diet (daily available ascorbic acid Ca 5 mg) for 7 days when the urinary excretion rose to 24 mg while blood ascorbic acid remained practically the same. This is interpreted as an indirect evidence of the influence of manganese in the synthesis of ascorbic acid.

17. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. K. P. Basu at the Dacca University, Dacca.

Protein content of soya bean and other pulses.

The protein contents of soyabean, green gram, dal arhar and Bengal gram are determined as 40%, 23.3%, 23.6% and 23.8% respectively.

Biological value of the proteins by the balance sheet method.

Biological values of the proteins of soya bean, Bengal gram, green gram and dal arhar determined by the balance sheet method have been found to lie within the range of 50–60% at 10 p.c. level of protein intake and their digestibility coefficients lie between 75–85%. From the experiments so far conducted it appears that the biological value of the proteins of soya bean and the other pulses for maintenance of nitrogen equilibrium in adult rats is of the same order. These experiments are being continued with a larger number of rats as suggested by the committee. Up till now only a limited number of experiments have been carried out.

Biological value by the growth method.

Growth experiments with a few young rats have shown that the biological value of the proteins of soya bean and the above mentioned pulses range between 0.8 to 1 gm. per gm. of protein intake at 10% level of protein intake. The mean values for the different pulses and soya bean, with six rats in each case were as follows :—

Soya bean	..	.. 0.86
Green gram	..	.. 0.97
Dal arhar	..	.. 0.95
Bengal gram	..	.. 0.80

Experiments with more rats will have to be carried out. The values for soya bean and Bengal gram are lower than those previously reported from this laboratory (1.5, 1.3).

Effect of supplementing poor rice diet.

Since soya bean contains a larger percentage of protein than the common pulses, the protein value which is the product of protein content, biological value and the digestibility is higher for soya bean than for the pulses. This higher protein value is likely to be reflected in the effect of supplementing soya-bean and the pulses on the poor rice diet. In order to determine the effect of supplementing soya bean and pulses on the growth of young rats, growth test with poor rice diet supplemented with the same quantity of different pulses and soya bean was conducted on groups of six young rats, weighing between 40–50 gms.

The mean weekly increases in body weight were as follows :—

	gm.
Rice diet alone ..	.. 1.85
„ „ +soya bean	.. 2.75
„ „ +dal arhar	.. 2.63
„ „ +green gram	.. 2.61
„ „ +Bengal gram	.. 4.5

These experiments require to be carried out with a larger number of rats.

18. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

The sub-committee on soya bean recommended, at its meeting held on the 16th December, 1941, that animal experiments to investigate the nutritive value of soya bean should include, (1) the determination of the biological value of soya bean protein and also of the other pulse proteins by (a) metabolism and (b) growth methods;

(2) the growth tests for the supplementary food value of soya bean and pulse proteins.

The technique of the experiments, the materials to be used and the treatment of results were discussed by the sub-committee and agreement was arrived at regarding the conduct of these experiments.

There was some delay in obtaining the materials after the scheme was sanctioned by the Governing Body of the Indian Research Fund Association. Consequently experiments were commenced somewhat later than expected. The assay of the biological value of the proteins under investigation by the balance method has been completed ; so also is the experiment of supplementary food value of soya bean and pulses. The assay of biological value by growth method has been commenced. The results will be submitted to the statistician when all the above mentioned experiments are completed.

19. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. Bashir Ahmad, Professor of Organic and Biochemistry, Punjab University, Lahore.

The appointment of the research chemist was made on the 8th August, 1942. The enquiry has therefore been in existence for less than three months. The soya bean sub-committee had suggested the following plan of work :—

1. The biological value of soya bean proteins by metabolism experiments.
2. Growth tests with soya bean.
3. Human experiments with soyabean.

A certain amount of the work has been done so far on the determination of the biological value of soya bean protein by the metabolism and growth methods. The metabolism experiments have been completed on four animals using the nitrogen balance method. The results of biological value lie between 73.6–74.7. These experiments, however, are preliminary and are being continued on another group of six animals.

The growth test on a group of animals receiving soya bean has been concluded. The value of growth per gm. of protein intake was found to lie between 1.3–1.54 with an average figure of 1.4. The results of other groups are not yet available. Even this test is being repeated on a fresh batch of animals, along with other groups.

Work on the effect of supplementation of a poor rice diet with soya bean and pulses has not yet been started.

A controlled experiment on school children has just been started in a local school with the cooperation of the Resident Medical Officer and the authorities of the institution.

It is hoped that by the end of this financial year the experiments will be concluded.

LEPROSY.

20. Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta.

IMMUNOLOGICAL STUDIES.

Our previous work on this subject had indicated that (a) the Mitsuda test even with antigens standardised by rough bacillary count had various limitations, (b) it was possible to isolate completely the bacilli from the leprous tissue, and (c) from these isolated bacilli it was possible to prepare chemical fractions. It was hoped that one of these fractions might be a specific antigen.

During the present year the work consisted of completing certain observations on the classical Mitsuda test with a standardised "antigen", improving methods of standardising bacillary antigen, and studying the protein antigen isolated from the bacilli.

Mitsuda reaction—(a) The Mitsuda reaction had previously been studied in different cases of varying types. The test has now been studied in the same cases under different circumstances. Major variations in results of the test were not observed; positives rarely become negative and no negatives became positive. Considerable variation, however, was observed in the degree of reaction in the same positive case in different circumstances. The two most important factors influencing these variation are (i) variations in clinical activity of the lesions and (ii) variations in the time of the year at which the test is done. With decrease in clinical activity the reaction tended to decrease. In the summer months even in the absence of variations of clinical activity, the reaction tended to become stronger. There is some evidence that allergic skin reactions are influenced by climatic conditions, and this may help to explain the clinical and immunological variations at different seasons.

(b) An attempt was made to confirm the reports of other workers that on repeated testing, an increase in the degree of reaction was observed. Monthly tests for a period up to eighteen months have failed to enhance the reaction to lepromin in nearly all the negative and weak positive cases tested, and the reports of other workers have not been confirmed.

2. *Attempts to isolate and study a specific antigenic fraction.*—This work has been facilitated by the use of a simple bacterial ball mill. The last report stated that 'by extracting the bacillus with different solvents, three different protein fractions have been isolated, and all of these are antigenic in cases of leprosy. There is a possibility that one of these fractions will be found specific". The three protein fractions were (a) acid-soluble protein, (b) nucleo-protein and (c) alcohol-soluble protein, which all gave good reactions of allergic type in neural cases of leprosy but the nucleo-protein was the most highly antigenic. When tested, however, in non-contacts in Punjab villages, none of these fractions gave entirely negative results. (The proportions of positive results obtained in non-contacts with the nucleo-protein and other preparations tested are recorded below.) An improved method of isolating protein was therefore tried, the phosphate-buffer method of Hiedelberger and Kendall. The nucleo-protein isolated by this method gave a lower proportion of positive results in non-contacts but it still appeared not entirely specific.

A study of the proteins of other acid-fast bacilli was started. Protein fractions similar to the above were isolated from the bacillus of Stefansky and

their antigenic properties were compared with those of similar fractions of the Hansen's bacillus. While the other two protein fractions of both bacilli appeared to act similarly, the nucleo-proteins acted differently, which fact suggested that any specific factor might be of this nature.

3. *The use of an improved bacillary antigen*—In view of the failure to demonstrate an entirely specific fraction of the bacillus, and in view of the fact that improvement in results was not commensurate with the extra work involved in preparing the protein fractions, a study of the antigenic action of the whole bacilli was resumed. It was found that the bacilli isolated and partly defatted by the use of chloroform gave a marked early reaction of allergic type as well as a late Mitsuda reaction and it was thought that by careful standardisation by weight and careful regulation of doses, the specificity of the test with this bacillary antigen might be increased sufficiently to make it of diagnostic value. Tests with this antigen were therefore made in non-contacts and a very low proportion of positive results obtained.

Results in non-contact adults.

Preparation	Approximate protein content of dose	Percentage of positive results in adult non-contacts
Ordinary lepromin	?	59
Ground bacilli	.005 milligram	60
Nucleo-protein alkali method	.01 „	75
Nucleo-protein phosphate buffer method	.01 „	36
do. do. do.	.002 „	5.5
Partly-defatted bacilli	.001 „	25
do. do.	.0002 „	3

All the above preparations in the above doses gave definite early reactions in most leprosy cases of neural type.

Conclusion—While with the improved and purer antigens used in recent work the percentage of positive results obtained in non-contacts has fallen markedly, a specific test for leprosy has not yet been evolved. The test however even in its present form may be of practical value. Moreover the work so far done gives clear indications of the existence of a specific antigen, and encourages further attempts to isolate it more completely or in a more natural form.

4. *Culture work*—An attempt was made to grow the bacillus of Stefansky in glycerine-broth-agar etc. containing the products of breaking down of the Stefansky bacillus by grinding. No definite multiplication of the bacilli was observed.

Epidemiological studies.

(a) *The Bankura investigation centre*— The work of this centre has been continued. The results of four years' study were given in last year's report. The observations are being continued.

(b) *Other epidemioloical studies*—Dr. Isaac Santra working under the Indian Council of B. E. L. R. A. has continued accurate leprosy surveys of the whole population of selected small areas. This year he has worked in one area

in Orissa and two areas in the Central Provinces and several areas in Madras, only one of which has yet been reported on. The main findings of these surveys are summarised in the table below :—

Area	Population studied	Incidence percentage	Type-distribution percentage of lepromatous cases	Age-distribution percentage aged 15 and under
1. Salem ..	6340	1.13	11	18
2. Kurud ..	5498	1.16	20	14
3. Kajikhed ..	4880	4.84	8	24
4. Khurda ..	5727	1.53	12.5	30

The following comments may be made :—Much has been heard of leprosy in areas 1, 2 and 4 but little of leprosy in area 3. Nevertheless area 3 shows much the highest incidence. Secondly the idea that a higher proportion of lepromatous cases usually goes with a higher proportion of cases in children is not supported by the above findings. General experience indicates that in some areas it is so but in some areas it is not so. A definite history of contact with an infectious case was often lacking, particularly in Khurda. Three possibilities have to be borne in mind. Neural cases may be more often infectious than is generally realised. This is however not considered highly probable. Secondly, casual or unknown contact may transmit the disease more often than is generally recognised. This is considered not improbable. Thirdly there is a remote possibility that the disease is transmitted by methods other than contact.

Animal work.

Six splenectomised and twice-inoculated monkeys have lived nearly three years since the first inoculation and remain healthy, and also the lepromin test in these monkeys on two occasions gave negative results.

Sundry work.

Observations on seasonal variation, on clinical variation in the disease over long periods and on associated immunological and histological changes are being continued.

21. Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras.

This enquiry was chiefly concentrated on epidemiology, certain aspects of immunity and the correlation of clinical types with the histological changes. Animal experiments and therapeutic experiments were also continued. Owing to the unique opportunity presented by the Child Investigation Unit at Saidapet, particular attention was given to child leprosy. All the material at the Lady Willingdon Leprosy Sanatorium and its associated clinics was available for research purposes.

Epidemiology.

Epidemiological studies were conducted at the Silver Jubilee Children's Clinic, and particular attention to epidemiology in relation to the control and spread of leprosy was given at the rural leprosy centre 23 miles south of Chingleput.

Epidemiological investigation at Saidapet.—During the year under report, fresh surveys were undertaken in three areas of Saidapet. A total population of 4,660 was examined. Out of these 3,158 were from an area comprising mostly of weavers. The remainder were in other areas whose occupations were varied but where only a few were employed in the weaving trade. The incidence of the disease in the three different areas is found in Table I, in which these three freshly surveyed areas are compared with areas earlier surveyed viz. area A and Mambalam village. It was found that the incidence of the disease was high in areas A and B.

Areas A and B are chiefly populated by the weaving community. Mambalam village is an exclusively Adi-Dravida community. Eddapalayam and Srirampet area also constitute an isolated colony but populated by different communities. The Fenpet area is one in which the percentage of Hindus and Muslims is approximately equal (54.6% Hindus and 45.4% Muslims). It is of interest to note that in areas A and B the open case rate is comparatively low, 24.8% and 16.7% respectively. In the Mambalam village, the open case rate is 14.3% which is very nearly equal to that in area B but the incidence is appreciably lower. The reason for this may be that the open cases from elsewhere do not mix with the Adi Dravida colony in this area, whereas in the other areas there is more promiscuousness. Among the open cases in Mambalam village since the survey was completed two have died, one is isolated in Lady Willingdon Leprosy Sanatorium and one is an occasional visitor. Actually only one is now residing in the village. In contrast to this, in areas A and B the movement of the open cases from place to place is so frequent and free that the chances of the spread of the disease are considerably increased. Hence the incidence in these two areas is higher in comparison to the other areas. In the two other areas populated by different communities it will be seen that the gross incidence of the disease is comparatively low. This may be due to the fact that there are few chances of mixing among the population in these areas since they belong to different communities.

Table I.

Comparative study of survey findings.

	Areas previously surveyed.						Fresh areas surveyed.								
	Area A			Mambalam village.			Srirampet & Eddapalayam.			Area B			Fenpet		
	Total.	Male.	Female.	Total.	Male.	Female.	Total.	Male.	Female.	Total.	Male.	Female.	Total.	Male.	Female.
Total population—Adults ..	1417	706	711	690	349	341	694	334	360	2116	1135	981	432	227	205
Number examined—Adults ..	1283	624	659	553	244	309	574	263	311	2071	1097	974	380	202	178
Total population—Children ..	794	410	384	413	214	199	398	226	172	1093	557	536	250	123	127
Number examined—Children ..	757	383	371	405	207	198	358	194	144	1087	553	534	210	100	110
Number of cases detected—Adults ..	70	37	33	16	9	7	25	8	17	100	67	33	9	5	4
Number of cases detected—Children ..	67	35	32	12	10	2	7	6	1	91	49	42	6	4	2
Percentage of Population Examined ..	96.7%			86.9%			96.1%			98.4%			100.0%		
Gross incidence ..	67.1	per	1000	29.8	per	1000	35.0	per	1000	60.4	per	1000	25.4	per	1000
Child incidence ..	88.5	per	1000	29.6	per	1000	20.7	per	1000	83.0	per	1000	28.57	per	1000
Sex incidence—Male ..	71.3	per	1000	42.0	per	1000	30.6	per	1000	70.3	per	1000	29.9	per	1000
Sex incidence—Female ..	63.1	per	1000	17.8	per	1000	39.6	per	1000	49.7	per	1000	20.8	per	1000
Sex Rate ..	52.5%			67.8%			43.75%			60.73%			60.0%		
Child Rate ..	48.8%			42.8%			21.9%			47.64%			40.0%		
Open case rate ..	24.8%			14.3%			12.5%			16.7%			26.6%		

The following points in the survey are of further interest. In four out of the five areas surveyed the child rates is 40% or more. This is, as is expected, associated with a high gross incidence except in the Fenpet area. The explanation of this may be that the open cases in this area have infected a large proportion of children in their own families. It will be of interest to see whether the comparatively high open case and child rate will result in the next few years in an appreciable increase in the incidence of the disease. In the new area surveyed (styled area B) out of the 91 children found to be suffering from leprosy 63.7% had been registered at the Silver Jubilee Clinic. This is an indication that the people of Saidapet are interested in leprosy and that there is comparatively little concealment of the disease.

Figures were published three years ago indicating that contact appeared to be the most important single factor in the acquirement of leprosy. Our work since then has continued to emphasise this. From an up-to-date analysis of nearly three hundred children the following points which emerge are of interest. The open case contact in 69.7% of cases was traced to an intra-familial or house contact. In 167 cases where the contact was maximal, that is, room contact, 32.3% developed the more benign form of the disease. The latter figure is of interest for it indicates that, even under the conditions most favourable for the acquirement of leprosy, one third of the cases apparently develop only benign forms. A further analysis therefore of the family history of these 167 cases has been undertaken. In 141 of these, satisfactorily accurate details were available. It is of interest to note that seventy open cases infected these 141 children, but that the total number of children exposed to the infection by close and intimate contact was 225, i.e. 62.7% developed the disease. The remaining 37.3% had no signs of the disease, but it is admitted that some of these cases may still develop leprosy in later years. It is stressed that surprise is expressed not at the number of persons who acquire leprosy, but that in spite of innumerable chances of infection, so few do develop serious leprosy. It is this relatively frequent occurrence of benign lesions in man which do not tend to develop into the more serious infective forms of leprosy which constitutes the greatest reason for optimism in dealing with the possibility of its ultimate control.

In order to estimate whether the factor of family susceptibility plays any significant part in the epidemiology of the disease, a complete analysis of 99 families consisting of 259 children and 311 adults was undertaken. Out of 259 children in varying contact with leprosy 143 or 55.12% developed the disease. Of these 143 children who developed the disease the source of infection was traced, and the following results ascertained :—

Percentage of children infected by	
Fathers	18.9%
Mothers	8.4%
Maternal uncles and aunts (inclusive of those by marriage) ..	9.1%
Paternal uncles and aunts (inclusive of those by marriage) ..	14.7%
Brothers and sisters	20.3%
Cotenants	28.6%

On the other hand the percentages of children with leprosy among those exposed to infection from these different sources are shown below :—

18 fathers have infected 27 out of 47 children or	57.4%
8 mothers have infected 12 out of 20 children or	60.0%
9 maternal uncles or aunts have infected 13 out of 28 children or ..	46.4%
16 paternal uncles or aunts have infected 21 out of 34 children or ..	61.8%
18 brothers or sisters have infected 29 out of 50 children or ..	58.0%
30 cotenants have infected 41 out of 80 children or	51.2%

Where there was a history of multiple contact in children the nearest relative was considered the probable source of infection. It may be of interest to note that out of 43 children who showed multiple contact 30 developed leprosy, that is, 69.75%. Whichever way these figures are interpreted it is difficult to trace any relationship between them and family susceptibility.

The comparatively high rate of infection from cotenants is probably explained by the fact that cotenants are likely to come into contact with a larger number of children. For instance, an open case in one family may infect the majority of the children in the family but that same open case may move freely among all the persons in the village or street and thus come into contact with a larger number of children. Therefore from the public health aspect such a source of infection may prove as important a cause of the spread of leprosy in the street or village as an open case within the family. This aspect of epidemiology needs to be stressed.

(b) *Epidemiological investigation at the Rural Leprosy Unit*—Routine survey of the surrounding villages has continued throughout the year, but of particular interest is the resurvey of our villages and three Adi-Dravida colonies within a period of three and a half years. The total number of new cases which have been discovered is 42. An analysis of these cases is being compiled with a view to publication. Table II is a comparison of the new cases in relationship to the effectiveness of segregation and the open case rate.

It will be noted that the highest incidence is in Perambakkam Adi-Dravida colony and yet only two cases have arisen and the percentage of open cases which have been under night segregation is the highest. In Polambakkam Adi-Dravida colony 3 cases have arisen but the open case rate is only 13.6%. Whereas in Perambakkam village where the open case rate is 35.3% only 4 new cases have declared themselves. Whether the relatively small number of new cases in the two villages where the segregation has been fairly effective is due to this or not, time only will reveal. In the village of Mukundagiri no new cases have declared themselves, even though this village is less than two furlongs from Perambakkam village and less than half a mile from Perambakkam Adi-Dravida colony.

Table II

Village	New cases	Percentage of gross incidence	Percentage of open cases	Percentage segregated over ten months Jan. to Oct. 42	Population
Polambakkam village	17	52.89	23.25	63.92	1,891
Polambakkam A. D. colony	3	44.0	13.63	61.98	1,023
Perambakkam village	4	46.7	35.29	87.87	871
Perambakkam A. D. colony	2	60.6	37.50	98.65	272
Maluvankaranai village	8	48.1	29.41	nil	742
Maluvankaranai A. D. colony	8	56.33	30.77	nil	51
Mukundagiri village	nil	14.57	nil	nil	706

IMMUNOLOGICAL INVESTIGATION.

Lepromin reaction—Eight hundred and fifty tests at the Silver Jubilee Clinic have been done during the year. This reaction was studied on the following lines :—

1. Lepromin in relation to age.
2. Factors tending to produce a negative lepromin.
3. Change of strength of the reaction in the same individual.
4. Lepromin in relation to progress of the disease.
5. Lepromin results at the Lady Willingdon Leprosy Sanatorium.

(1) *Lepromin in relation to age*—It has been suggested that a positive lepromin is not seen in children under 4 years. The youngest child in the following series was 3½ years of age. The analysis below suggests that the lepromin reaction has little relation to age at least in children over the age of five.

Table III.

	5-9			10-14		
	No.	Negatives	Percentages	No.	Negatives	Percentages
Nl Ns ..	25	10	40.0	42	19	45.2
Nl Nt Minor & Major ..	44	5	11.4	39	3	7.7
Incipient ..	9	4	44.4	10	6	60.0
Lepromatous ..	6	6	100.0	14	14	100.0
Suspicious & Healthy ..	26	8	30.8	19	6	31.6

(2) *Factors tending to produce a negative lepromin*—The evidence that is accumulating tends to show that whereas the positive lepromin reactions show little significant variation between a family which has a history of leprosy and persons of a family without such a history, in those with extrafamilial and chance contact the average positive rate is nearly twice as much as in those with intrafamilial contact. Thus we feel that it is legitimate to maintain our conclusion that 'family susceptibility' is not an important factor in breaking down the lepromin reaction. Table IV gives support to this contention for it demonstrates that there is not a significant difference between the lepromin reaction in those with a family history of leprosy and those without such a history.

Table IV.

Percentage of lepromin positive in relation to history of leprosy in the family.

	No. Done	Positive	Percentage
Family history ..	96	67	69.7
No family history ..	98	71	74.1

(3) *Change of strength of the reaction in the same individual*—Hundred and sixty re-tests in hundred and forty children were done. Thirtyone negative lepromins in neural and incipient cases became positive. But at the Silver Jubilee Clinic no positive lepromin was found to become completely negative. In 7 tuberculoid macular and 2 simple neural cases, the lepromin changed from a higher to a lower degree of positivity, i.e. 4 plus or 3 plus to 2 plus or 1 plus but none became totally negative. In one lepromatous case which was bacilli free for over a period of 3 years the lepromin was once 1 plus and later became negative.

(4) *Lepromin in relation to progress of the disease*—There appears to be some relationship between the progress of the disease and lepromin reaction. In the following table it is seen that among the neural and incipient cases 54% which became worse had a negative lepromin whereas only 12.4% had a negative leprmin in those which had improved.

Table V.

Total	Improved	Percentage lepromin negative	Stationary	Percentage lepromin negative	Worse	Percentage lepromin negative
233	129	12.4	54	27.7	50	54

(5) *Lepromin results at the Lady Willingdon Leprosy Sanatorium*—Two hundred and eightythree tests have been done during the year. To date no active lepromatous case has shown a positive reaction ; one case previously diagnosed as leproma, but now negative to standard methods of examination, has shown a weak positive (1+). Another case previously classified as major tuberculoid in a state of acute reaction returned to the sanatorium after an interval of two years and five months. The lesions have changed their clinical appearance and the lepromin which was previously 2+ is now negative. This latter case is of great interest and is being studied in more detail. Apart from this the results of the lepromin at the Lady Willingdon Leprosy Sanatorium are substantially the same as those at the Silver Jubilee Children's clinic.

Development of lesions.

A study of the development of lesions was continued throughout the year. An assessment of the progress of children was made. Out of a total of 204 children on observation only 23.5% (among simple neural), 6.5% (in tuberculoid minor), 4% (in tuberculoid major) and 66.3% (in the incipient groups) showed deterioration while 55.9%, 64.1%, 64% and 27.8% respectively showed improvement. This confirms the opinion that approximately 50% of neural leprosy among children may be benign.

Regarding the development of lepromatous leprosy 5.74% of simple macular cases became lepromatous during the past 5 years while 25.7% of the cases in the incipient category developed lepromatous leprosy. This emphasises the comparative seriousness of the latter group.

Clinical and histological work.

The correlation of histological findings with the clinical characteristics has been continued throughout the year and the intensive study of atypical

lesions pursued. This group known as the intermediate or border line case is resolving itself in S. India into several categories as viewed from the histological point of view. Detailed study of these lesions is being pursued and particular attention is being paid to their development and prognosis. A number of interesting findings are being recorded and a more detailed report on these will be furnished when the histological material has been further studied.

Therapeutic experiments.

These have been continued as in previous years. New remedies, both indigenous and western were tried with no outstanding results. The enquiry to discover the optimum dose of hydnocarpus oil has been continued, as also a trial of the effectiveness of Avenyl in tending to make a positive Wasserman reaction negative is being undertaken. It is felt that the postion with regard to leprosy and syphilis needs to be re-investigated, for we have not been able to confirm the conclusion of past workers as to the great importance of this disease in the treatment and development of leprosy.

Animal experiments.

Animal inoculation experiments are being continued in cooperation with the staff of the King Institute, Guindy. While we still have to report failure to produce progressive leprosy in any animal, work is being pursued in connection with the lepromin test and the relationship of this reaction to repeated inoculation. No detailed report is presented of this work, for an explanation of certain contradictory findings has yet to be sought.

PLAGUE.

22. Plague researches under the Director, Haffkine Institute, Bombay.

Field trials of combined treatment with sulphathiazole and antiplague serum.

The plague hospital built by H. E. H. the Nizam's Government at Latur was again utilised for this trial. The trial lasted from December 1, 1941 to March 31, 1942.

Total cases of plague treated		140
Excluded from reckoning—		
Moribund at the time of admission ..	25	
Leaving hospital against advice.. ..	2	
Pneumonic plague	2	29
		111

Treatment and dosage—Admissions were treated in rotation with sulphathiazole *cum* antiplague serum and sulphathiazole alone. Sulphathiazole was given in dosage of 10 g. the first day and 7.5 g. a day for the subsequent 3 or 4 days with reduced dosage later. In continued treatment the dosage of sulphathiazole was kept the same and 40 cc. serum a day for 4 or 5 days was given in addition.

All Cases.

Treatment	Number of cases	Deaths	Case mortality per cent
Sulphathiazole ..	51	10	19.6
Sulphathiazole plus antiplague serum ..	60	12	20.0
Total ..	111	22	..
Previous controls ..	149	86	53

Cases with plague septicaemia at the commencement of treatment.

Treatment	Number of cases		Case mortality per cent
Sulphathiazole ..	18	9	50.0
Sulphathiazole plus antiplague serum ..	25	8	32.0
Total ..	43	17	..
Previous controls ..	75	68	90.6

The total number of cases treated is small and the results are not significant statistically but clinically a marked advantage was noticed in favour of the combined treatment. Toxic symptoms disappeared much sooner, within a few hours, in cases which received both serum and sulphathiazole, while among cases treated with sulphathiazole alone, these lasted for 3 or 4 days. This rapid clinical improvement was a very marked feature of this trial. Further trial is necessary to put this noted difference on a statistical basis.

STUDY OF THE FACTORS WHICH AFFECT THE PROTECTIVE POWER OF THE VACCINE.

1. *The relationship of the virulence of a strain to the protective power of the resultant vaccine*—There has been considerable uncertainty on this point, but the problem could not be tackled until we could, at will, reduce progressively the virulence of a given strain. This has now been achieved. The results given below are not only interesting in themselves, but show the way to produce and select the best possible avirulent strain for the production of a live vaccine.

Subcultures of strain 53/H	Virulence (measured as M. L. D. for a mouse)	Immunogenic value (measured as mouse protective dose)
1st	5 organisms ..	Indeterminable
15th	25 organisms ..	6 organisms
25th	300,000,000 organisms ..	875 organisms
31st	600,000,000 organisms ..	3,850 organisms
36th	1,500,000,000 organisms ..	11,250 organisms

2. *Comparison of the immunogenic value of a number of avirulent strains*—Otten's (Tjiwidej Smooth) and Girard and Robic's (E. V.) avirulent strains are the classis strains. They were found accidentally, but now that we have found a method of reducing at will the virulence of a strain and can measure its immunogenic value during the process of attenuation, it makes it possible to select a suitable strain at will for preparing a live vaccine. The comparative measurements given below show that our 25th sub-culture of strain 53/H is sufficiently avirulent and yet much more immunogenic than the classic strains.

Strain.	Mouse protective dose.
Otten strain (Tjiwidej Smooth)	800,000 organisms
Girard and Robic strain (E.V.)	800,000 organisms
Haffkine Institute (53/H/25)	875 organisms

It is clear that the 15th sub-culture of strain 53/H is much more immunogenic (100 times) than either Otten's or Girard and Robic strains. It is also more immunogenic than the 31st and 36th subcultures of the same strains. We are continuing to subculture this strain further, and it seems that a point will be reached when it will lose its immunogenic power altogether. In fact, in the case of another strain, 65th subculture was found to have no immunogenic power at all. A number of strains (4) are being studied in the same manner.

3. *Live avirulent vaccines*—Last year, even working with less immunogenic avirulent strains, Otten's, Girard and Robic's and 53/H, we came to the

conclusion that the live avirulent vaccines are the most effective. Now that with the technique described in para 3 above, it is possible to select a still more immunogenic strain, the superiority has moved still further in favour of live vaccines. In terms of the notation employed last year, i.e. mouse protective dose of vaccines in c.cs., the results are as follows. Each vaccine contained 1000 million organisms per c.c. of agar-grown cultures.

Live vaccine	Mouse protective dose
Haffkine Institute (53/H/25)	0.000001 c.c.
Otten's Twijdej Smooth	0.001 c.c.
Girard and Robic's (E.V.)	0.001 c.c.

Killed vaccines	Mouse protective dose
Heat (54°C)	0.0065 c.c.
Merthiolate 1 in 500,000	0.0068 c.c.
Sulphathiazole—1%	0.0070 c.c.
Alcohol (Felix)	0.0160 c.c.

4. *Killed vaccines made from virulent and avirulent strains*— Though virulent strains, as shown above, are very much more antigenic than avirulent strains, yet when the cultures are heat killed the immunogenic value of both falls to about the same level.

	Strain	Mouse protective dose	
53/H/15	Virulent live	6	organisms
53/H/1	Virulent—heat killed (54°C for 15 minutes)	6,500,000	organisms
53/H/25	Avirulent—live	875	organisms
53/H/25	Avirulent—heat killed	5,300,000	organisms

These results are highly significant, and show that killing of the cultures even with the very minimum of heat needed to kill reduces the immunogenicity of the strain to five thousandth to a millionth of the original figure. The problem then is to find, if killed and preserved vaccines are desired, a better way of killing cultures. We have tried merthiolate, sulphathiazole and alcohol, ultra-violet light without success. However, further work on this important subject is being carried out, and we hope to present extensive results next year.

5. *Agar-grown and broth-grown vaccines*—Last year we reported important findings on this subject to the effect that the mouse protective doses of agar, heat-killed, vaccine (1000 million organisms per c.c.) and 4-week Haffkine broth vaccine were 0.0065 c.c. and 0.0060 c.c. respectively. Although the mouse protective doses of the two vaccines were about the same, yet the advantage lay with the agar vaccine, because it needed only 3 days' incubation period to make it.

We have carried this work further to a fruitful point. We find it is possible to produce as good a broth vaccine with a three-day growth as an agar vaccine with a distinct advantage in favour of broth vaccine. With this object in view, we experimented with different media and find that a casein hydrolysate broth (nitrogen content 270 mg per 100 c.c.) is the ideal medium when 600 c.c. of this

broth is dispensed in a 4-litre Haffkine flask, 3 days' incubation at 28°C, gives a growth which has 1000 million organisms per c.c. When dispensed in this manner, the surface area of the broth is 254 sq. c.m. Now, if agar is dispensed in the same flask with a surface area of 254 sq. c.m. 3 days' incubation at 37°C gives 160 c.c. of a suspension with 1000 million organisms per c.c. Thus broth, after 3 days' incubation, gives 600 c.c. of vaccine, while agar gives only 160 c.c. of the vaccine. The mouse protective dose of these vaccines—both heat-killed (54°C — 15 minutes) is almost the same as shown below :

Vaccines	Mouse protective dose
Broth vaccine—3-day growth (1000 million organisms per c.c.)	0.0053 c.c.
Agar vaccine—3-day growth (1000 million organisms per c.c.)	0.0048 c.c.

These experiments show that at least in the case of plague organism a good broth vaccine can be made up as rapidly as an agar vaccine, except that broth vaccine is obviously easier to make, and what is still more important at this time, no agar is required.

6. *Toxicity of different types of plague vaccine*—We have produced abundant experimental evidence to show that the protective power of Haffkine vaccine is as good as that of any heat-killed agar vaccine, but the toxicity of the Haffkine vaccine has been an objectionable feature. The three-day growth broth vaccine which we have made gets over even this difficulty.

Vaccine	Mouse protective dose	Toxic dose (Dose producing death of 50% of mice used)
Haffkine vaccine	0.006 c.c.	0.2 c.c.
New broth vaccine (1000 million organisms per c.c.)	0.0053 c.c.	0.8 c.c.
Agar vaccine (1000 million organisms per c.c.)	0.0048 c.c.	1.0 c.c.

SEROLOGICAL STUDY OF STRAINS.

1. A study of the serological differentiation of various strains, representing virulent, avirulent protective, avirulent non-protective and pseudotuberculosis groups was continued by means of agglutination and cross-agglutination, absorption and cross-absorption of agglutinins and precipitin tests using antisera raised against the individual strains in rabbits. In all, 16 strains with their corresponding antisera made against live organisms were used and more than 2000 sets of agglutination performed. The following observations have so far been made :—

By the agglutination tests *P. Pestis* strains could be divided mainly into two groups—protective and non-protective. The virulent and the avirulent strains constituting the protective group could not be distinguished from each other by these tests but the avirulent, non-protective and the pseudotuberculosis groups could be sharply differentiated. While all the four groups mentioned above were agglutinated by the antisera against the virulent or avirulent protective strains, the antisera against the pseudotuberculosis and avirulent non-protective strains did not agglutinate the protective strains, but they cross-agglutinated and cross-absorbed each other freely. When, however, an avirulent non-protective strain possessed a residual antigen corresponding to that of the protective strains, the latter were agglutinated only to a low titre, 1 : 25 or 50 by its antisera.

2. *Study of immunisation of horses*—In the process of immunisation of horses against the plague strains, sera from test bleedings at different stages were tested for agglutination reaction against the various groups of strains. It was noted that the surface antigen titre goes up parallel with the somatic titre for the first few injections but with superimmunisation the former remains practically stationary while the latter only goes up. The relationship of these two antibody fractions in the matter of protection against plague infection may be suitably studied along with the study of the antigenic analysis mentioned above.

3. *Synthetic casein hydrolysate medium*—This medium was further studied with regard to the effects of essential and supplementary nutrients viz. N, P, Ca, Mg, Fe, Cystine, Tryptophane, liver extract etc.

A modification was introduced in neutralising the excess acid with crude $BaCO_3$ instead of $Ba(OH)_2$. $BaCO_3$ is much cheaper and brings down the cost of the medium materially.

4. *Isolation of specific protein*—It has been found that the yield of specific soluble substance in cultures grown in casein hydrolysate medium is maximum after 3 weeks' growth at 28°C when the pH of the culture is about 8.4. This specific substance has been fractionated at different concentrations of Na_2SO_4 and these fractions will be extensively studied serologically, chemically and for their immunogenic value in animals.

A similar study extended to other strains viz. (a) virulent, (b) immunogenic avirulent, (c) non-immunogenic avirulent and (d) pseudotuberculosis will give an opportunity to classify the organisms on an antigenic basis.

5. *Standardisation of anti-plague sera*—Experiments were carried out to develop a suitable test tube method for evaluating the potency of horse sera at various stages of immunisation. The ordinary agglutination and precipitin reactions fail to serve the purpose. But the quantitative estimation of agglutinin nitrogen or precipitin nitrogen with standardised antigens seems to give a fairly good index to judge the potency of the sera. The antigen used in the former case was a standard formolised suspension of the organisms and in the latter the specific protein isolated from virulent plague cultures at one-third saturation of Na_2SO_4.

Ser. No.	Date of bleeding	Precipitin-N per c.c. in mgms.	Agglutinin-A per c.c. in mgms.	Mouse protective dose in c.c.
H27 (conc.)	..	0.94	..	0.17
H24	2–11–41	0.65	..	..
H21	,,	0.63	..	0.24
H45	,,	0.51	..	0.45
H23	,,	0.40	..	0.60
H27	,,	0.37	..	0.40
H44	,,	0.21	..	0.70
H23	28–11–41	1.36	2.04	0.075
H24	,,	0.96	1.24	0.18
H44	,,	0.68	0.60	0.29
H27	1–5–42	..	1.60	0.068
H23	1–5–42	..	1.28	0.084

TUBERCULOSIS.

23. Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta.

The researches carried out during the year under various heads are summarised below.

JUTE INDUSTRY.

The investigation was undertaken among the workers of the jute industry, as it is one of the main exclusive industrial enterprises of Bengal and as it emits dust in the industrial process (i.e. it is a " dusty industry "). The Angus Jute Mill was selected as the centre of investigation, as it is one of the biggest mills in Bengal equipped with X-ray plant and as good cooperation from the authorities was available. This industrial centre is situated 23 miles away from Calcutta and employs about 5,000 workers.

The general plan of investigation—The main purpose of this enquiry has been to investigate the prevalence of tuberculosis among mill workers, who are subject to the dust, dirt, noise and vibration of the factory during working hours. In order to assess the effect of this factory environment on the incidence of tuberculosis, four groups of population were investigated. These have been designated A, B, C and D viz., group A forms the population of mill workers while B consists of those who live in the mill area, i.e., in the industrial environment without being subject to the special strains of factory work; group C comprises those who live away from the industrial area but have intimate connection with the industrial population and group D of those who live in villages away from the factory area and have very little contact with the industrial population. It was thought that such planning of the survey was likely to indicate the effect of industrialisation in gradual degrees.: group D would show the tuberclosis status in a village population not connected with the industry ; group C in village people, who are in contact with industrial workers but are not living in an industrial environment, group B among people who are living in an industrial environment without being, at the same time, exposed to the stress and strain of factory work and group A in people subject to the influence of both the factory and the industrial environment.

As India is still in the infancy of industrialisation these different types of population can be demarcated fairly well. With the growth of industry, it is anticipated that these differences are likely to be obliterated. This was, therefore, in point of time an ideal opportunity to plan the investigation.

Group (A). Jute mill workers.

(i) Intensive study of sample workers from different departments of the factory—Altogether 263 workers were selected and examined by taking detailed history of life previous to and after entrance into the industry. Every worker was physically examined, and tuberculin tested. His blood was examined for Von Bonsdorf and lymphocyte : monocyte ratio counts, chest radiologically examined and whenever possible his sputum was examined for acid-fast bacilli.

It was detected that 80% of the workers came from rural, 18% from semi-rural and only 2% from urban areas. Workers labelled as " definite " cases of pulmonary tuberculosis formed 1.9% (sputum positive and/or radiologically definitely active infiltration in lung parenchyma) ; 5.3% were " probable "

cases (sputum negative or unknown but radiological study suggested the presence of active disease) and 59% were "arrested" or "healed" lesion cases (either in tracheo-bronchopulmonary glands or in lung parenchyma). The infection rate as detected by Mantoux test was 88.7%. Evidence of bronchitis was detected in 32%, jute dermatitis in 4% and 60% had a high eosinophile count in blood.

(ii) Serial examination of all workers—This investigation was carried on along the above mentioned lines except that, for financial reasons, only those workers who showed suspicious signs or symptoms of tuberculosis or of bronchitis and high tuberculosis reaction (3 and 4 pluses) were X'rayed. Blood tests were not done in this group. The number of workers examined under this group was 4,816. The infection rate, as determined by Mantoux test, was found to be 82.4%, of the 1656 selected cases which were X'rayed active pulmonary, tuberculosis was found in 112 or 6.8% and probable active lesions in 68 or 4.2% of the cases. Judging from these figures, active pulmonary tuberculosis cases in a total of 4,816 jute mill workers were found to be in 180 or 3.7%. In the X'rayed group, "arrested" lesions were detected in 85.4%. Jute dermatitis was detected in 5.6% of the workers.

(iii) Environmental condition of the factory, with special relation to size and concentration of dusts—Samples of dust were collected from different departments and at different seasonal periods. For the determination of concentration and size of the dust, the "dust counter" of Bausch and Lomb was employed. The counting was done by a built-in-compound microscope of 200 × magnification with a special dark field illuminating system attached to it.

It was found that the majority of the dust particles floating in the factory were below the size of 5 micron, about 90% of which were, between 1–3 micron in size. All the departments were not equally dusty. The highest concentration of dust between 7–10 million particles per cubic foot of air was detected in the 'breaker line'—a part of the preparing section. The next highest concentration was in the spinning section.

Group (B). Relatives of workers residing in the spinning section.

This investigation was carried on along the same lines as above, 1107 persons being examined. The rate of infection of tuberculosis was found to be 65.2%. In the selected X'rayed 240 cases 20, or 8.3% had 'definite' and 10 or 5.1% had 'probable' active pulmonary tuberculosis. These figures distributed over the total group showed that active pulmonary tuberculosis was likely to be present in at least 2.7% of the population.

Group (C). Relatives of workers residing in villages outside the mill area.

This investigation was carried on along the same lines as above, 1100 persons being examined.

The rate of infection of tuberculosis was found to be 67.6%. In the selected X'rayed 114 cases, 13 or 11.4% had 'definite' and 8 or 7.0% had 'probable' active pulmonary tuberculosis, or in the total population at least 1.9% were suffering from active tuberculosis.

Group (D). Village population not connected with industry.

This investigation was conducted along the same lines as above, and 1200 persons from villages about 5 to 7 miles away from the Angus Jute Works were examined.

The rate of infection in the villages was found to be 42.5%. Among the selected X'rayed 84 cases, 7 or 8.3% had 'definite' and one or 1.2% had 'probable' active pulmonary tuberculosis, or in the total village population at least 0.6% had active pulmonary tuberculosis in them.

Comparison of groups A, B, C, and D.

The following table sets out the rate of infection and disease in the four areas of investigations.

Survey area	Type of population	Infection rate	Disease rate
A	Mill hands exposed to factory and industrial environment.	82.4%	3.7%
B	Relatives of workers : exposed to industrial environment.	65.2%	2.7%
C	Relatives of workers : no industrial environment but intimate connection with mill workers. ..	67.6%	1.9%
D	Village population : rural environment ..	42.5%	0.6%

The above table clearly shows the influence of industry on the incidence of tuberculous infection and disease. The higher the degree of industrialisation, the greater was the incidence.

The sputa of X'ray 'definite' and 'probable' active tuberculosis cases from each type of survey, whenever available, were examined. The negative sputa were re-examined. The following table shows the result of these examinations in the different types of people.

	'Definite' active cases (Radiologically)			'Probable' active cases (Radiologically)		
Type	Number radiologically detected	Sputum examined	Number positive	Number radiologically detected	Sputum examined	Number positive
A	112	96	52	68	66	5
B	20	18	15	10	7	1
C	13	13	11	8	8	2
D	7	3	2	1	1	0
Total	152	130	80=61.5%	87	82	8=9.7%

It might be recalled that all the active tuberculosis cases diagnosed in these surveys were ignorant of their disease. They were, therefore, moving freely and spreading infection to their surroundings. As they were supposed not to be ill they were extremely reluctant to agree to have their sputum examined; hence sputa from all cases were not available. However, the above findings certainly show that a large number of 'open' cases are associating freely with the local people, specially in the industrial area. These areas are very crowded and hence the spread of infection is expected to be fairly intensive.

Home survey of the industrial and rural populations.

Among the environmental factors, living conditions are important for the causation and spread of tuberculosis. With this view 1746 homes of the workers of the mill and 220 homes of the village population (group D) were investigated. The homes taken up for survey were as follows :—in the mill and its neighbourhood ; barracks—1010, bustees—512, village huts—224 ; in village areas (D) ; masonry houses—28, semi-pucca huts—34 and kutcha huts—158. The sanitary condition of the rooms and the surroundings were unsatisfactory in almost every case, except in the case of barracks where the environmental sanitation is fairly satisfactory due to the continuous efforts of the mill authorities towards improvement of hygiene.

Crowding in the homes of the workers was to the extent of 4 persons per room of an average size of 13′ × 10′, and that in the homes in the contrast village was on the average of 4 persons per room of an average size 13′6″×11′3″. The percentage of tuberculosis cases detected in the homes varied in different types of houses as follows :—in barracks—8.1%, in neighbouring bustees—5.9%, in village huts in the neighbourhood of the mill—3.2% and in the homes of distant contrast villages—0.7%. The above figures indicate that the nearer a home is to the industrial area, the greater is the incidence of disease in its inhabitants. On further analysis, it has been noticed that the greater the crowding, the larger is the incidence of disease.

Survey of home contact cases, Group A—Five hundred 'home contacts' of the tuberculous workers of the mill (110 'definite' and 60 'probable') were examined and the results analysed. Of these contacts, 82.6% reacted to the tuberculin test. Among the selected 150 cases of this group 10 or 6.6% showed 'definite' and 7 or 4.6% 'probable' evidence of active disease in the lungs, or a total of 11.2% active disease in the selected group. In the whole group of 500 contacts, therefore, the incidence of diseases may be taken as at least 3.4%, a rate of incidence almost equal to that among the mill workers.

Group B—Among the relatives residing in the industrial area, 20 'definite' and 10 'probable' cases of pulmonary tuberculosis were detected. Their contacts, 128 in number, were examined. The rate of infection among them was found to be 87.5%. In the selected X'rayed 35 cases of this group 19 or 54.3% showed 'definite' and 9 or 25.7% 'probable' disease. Distributed over the whole group the incidence of active disease may be taken as 21.8%, an extremely high rate. It is possible that as these cases were generally unemployed and remained at home all the time, the incidence of disease was found to be so high among them.

Group C—Among the relatives residing outside the industrial environment, 13 'definite' and 8 'probable' cases of pulmonary tuberculosis were detected. 97 home contacts of these cases were investigated. The rate of infection in these contacts was found to be 82.4%. In the selected X'rayed 31 cases of this

group 'definite' tuberculosis was detected in 13 or 41.9%, and 'probable' in 8 or 25.8%. Distributed over the whole group the incidence of active disease may be taken as 21.6%. The reason for this very high incidence is probably the same as in group B.

Group D—Among the rural population of the contrast village, 7 'definite' and 1 'probable' cases of active pulmonary tuberculosis were detected; 74 contacts of these have been examined. The rate of infection among them was found to be 71.6%. In the selected X'rayed 30 cases of this group, 'definite' tuberculosis disease was detected in 6 or 20% and 'probable' in 2 or 6.6%. In the whole group, therefore, 10.8% may be presumed to be suffering from active disease — a very high incidence indeed.

The following table contrasts the rates of infection and disease in the general population and in the home contacts of active pulmonary tuberculosis cases of the different groups :—

	Incidence of infection		Incidence of active pulmonary tuberculosis	
Group	General population	Home contacts	General population	Home contacts
A	82.4%	82.6%	3.7%	3.4%
B	65.2%	87.5%	2.7%	21.8%
C	67.6%	82.4%	1.9%	21.6%
D	42.5%	71.6%	0.6%	10.8%

The above table clearly shows the disastrous effect on the home contacts of active tuberculosis cases of these areas where housing and sanitary conditions are very unsatisfactory. The rate of infection and specially that of the disease show a very high figure among the contacts. In group A (mill hands), the rates are almost the same in the general and contact population. The reason for this may be that the mill workers stay out of home all day and are, therefore, less frequent sources of infection and of disease in the homes.

Follow-up survey.

(i) *Mantoux negative cases*—Out of 846 mill workers who were negative to the first Mantoux test, 612 were retested after a period of about 2 years (first with 1/1000 O.T. and then, if negative, with 1/100 O.T.). Among them 533 or 87.0% proved positive.

(ii) *Diagnosed pulmonary tuberculosis cases*—Seventyeight tuberculosis cases (48 'definite' and 30 'probable') were followed and re-examined. Out of these, 63 remained stationary, 13 became worse and only 2 improved.

Bata shoe factory.

This factory has a labour strength of about 7,507. It is one of the most modern large industries in Bengal. The workers are housed better and the

factory environment does not contain so much dust as in the jute industry. The investigation into this modern industry is, therefore, likely to make a good contrast with that of the old jute industry.

As the time for conduction of this survey was very limited, only a sample survey was initiated. About one-fourth of the workers of each of the 10 departments of the factory were selected as representative samples for investigation.

The investigation is being carried out along the same lines as that in the jute industry. Of the 508 workers who have been examined, 447 workers of the Rubber Rolling and Heal Pressing and Rubber Shoe manufacture (in part) departments have been put through all the tests (except chest skiagraphy).

The age groups of these workers were as follows :— 15 years—9, 20 years—134, 25 years—135, 30 years—103, 35 years—46, 40 years—20.

Out of these 447 workers, 360 or 80.5% proved positive to Mantoux test (+=121, ++=114, +++=118, ++++=7). The infection rate according to age periods were found to be as follows :—15 years—77.7%, 20 years—77.2%, 25 years—76.3%, 30 years—88.3%, 35 years—86.9%, 40 years—80.0%.

By physical and other examinations, 7 were found to have suspicious signs and symptoms of pulmonary tuberculosis, 39 showed evidence of bronchitis and 125 were high tuberculin reactors. The number of workers showing suspicious indications of tuberculosis and/or bronchitis and/or high reaction to tuberculin test was 158. These have been selected for X'ray, which is being done.

The investigation is still proceeding.

Tuberculosis among home contact children.

A total of 1125 contact children traced from definitely diagnosed cases attending the chest department of the Medical College Hospital, Calcutta are included in this investigation. Of these, 1013 were tested with tuberculin (1/1000 O.T.) and 877 were examined radiologically. Of the former 65.1% reacted to tuberculin and of the latter 40.5% showed radiologically demonstrable pulmonary lesions (10.4% 'active' lesions, 4.8% 'arrested' lesions in the pulmonary parenchyma and 25.3% tracheobronchial adenopathy).

It has been found that the average incidence of infection (tuberculin positive) varies *directly* while that of demonstrable X'ray lesions *inversely* with the age of the contacts. The incidence of active parenchymal lesion (disease) decreases while that of arrested parenchymal lesion increases with age. The incidence of tracheobronchial adenopathy increases with age upto 5 years and then decreases.

The rate of infection and disease differs very little between the different sexes and communities.

The number of urban (954) and rural (117) domiciles were not at all comparable. Still the findings show that both infection and disease are greater in urban (65.2% and 11.0% respectively) than in rural children (37.9% and 9.0% respectively).

The majority of these children (821 out of 1,125) were exposed to positive sputum cases. These children gave a higher incidence of infection (67.5%) and demonstrable radiological lesions (9.4% 'active', 6.1% 'arrested' and 28.7% tracheobronchial adenopathy), than those exposed to negative sputum cases (53.7%), 4.0%, 0.8% and 16.2% respectively). The children exposed to

'unknown' sputum cases, however, showed the highest incidence of active lesions (20.4%). This is probably due to the usual absence of care about contact with the sources of infection and the disposal of sputum in these cases.

Among the children exposed to 'open' or positive sputum cases the incidence of both infection and disease were far greater in the *intimate contact* group. (70.3% and 9.9% respectively) than in the *distant contact group* (43.3% and 2.3% respectively).

The duration of contact with positive sputum cases was recorded from the history related by the children's relations who naturally calculated it from the time of diagnosis or when serious illness was apparent. The duration is, therefore, much shorter than the actual duration as the source was probably already infectious many weeks or months earlier. With 6 month's duration, the infection rate was 48.1% and the disease rate 0.9%, whereas when the duration was 2 years or more the corresponding figures were 75% and 18.5% respectively. This points out that the incidence of both infection and disease increases with the duration of contact.

Among children in contact with open cases the maximum number (30.0%) had contact with tuberculous mother and the incidence of disease in this group was highest (11.8%). This indicates that the contact of a young child with the tuberculous mother is the greatest source of danger.

Altogether 87.5% of children with 'active' and 'arrested' parenchymal lesions, 72.9% with tracheobronchial adenopathy and 63.0% with no demonstrable lesion reacted to tuberculin (1/1000 O.T.). On the other hand 29.7% of negative reactors showed some kind of radiologically demonstrable lesion.

The incidence of intense tuberculin reactions (3+ and 4+) was highest (33.3%) with active disease and lowest (20.6%) with no demonstrable lesion cases. Again 13.1% intense reactors against 11.2% mild ones showed active disease. These findings indicate that a greater incidence of active disease is expected in the case of intense reactors.

Home Survey—Many of these children have left their addresses since registration at the clinic, while a number of addresses could not be traced. Altogether 230 homes have hitherto been surveyed. Environment was unsatisfactory in most cases. Many of the homes were of the bustee and barrack type, a small proportion occupied the ground floor of insanitary buildings, and only a few were found in well ventilated and sanitary buildings. In most of the houses isolation of children was not observed. Overcrowding was prevalent especially in one-roomed tenements where on an average 5 persons lived in a room whose average size was 12′ × 9′. The incidence of the disease (active parenchymal lesion) was found to vary with crowding but the infection rate (as elicited by tuberculin reaction) did not show any such correlation. Economic status showed no relation with the incidence of tuberculosis among the contacts.

Typing of tubercle bacilli from pathological materials.

Altogether 254 strains have been isolated from different varieties of pathological materials as noted below. Cervical gland—67, axillary gland—7, inguinal gland—1, mesenteric gland—13, bones and joints (cold abscess)—21, tonsils—2, skin—5, urine—4, stool—1, stomach wash—1, cerebrospinal fluid—1, pericardial fluid—1, pleural fluid—36, tracheobronchial gland—37, lung tissue—4, sputum—53. The strains were isolated by culture and animal inoculation with the materials, after preliminary treatment with 6% H_2SO_4 wherever necessary. The age

incidence of the cases from which these materials were obtained ranged from a few months to very old age. For typing, isolated strains were passed on to rabbits and guineapigs, in addition to noting their cultural characters. The cultural characters and the animal inoculation experiments proved that all the strains belonged to the human type. The result of typing of the first 60 strains were reported in the I. J. M. R. Vol. XX, No. 4, April, 1933, These findings indicate that the part played by the *bovine* type of tubercle bacilli in the causation of human tuberculosis in this country is negligible and not at all important.

PHARMACOLOGY.

24. Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore.

During the year the following indigenous drugs were investigated :—

Rasaut.

It is a solid watery extract prepared from roots of various species of berberis. It is a common indigenous medicine. Analysis of 15 samples showed that the percentage of berberine in various samples sold in the market varies from 0 per cent to 4.2 per cent. If toxicity of a sample of *Rasaut* is determined on frogs and compared with the toxicity of the calculated amount of berberine in it, our work has shown that *Rasaut* was more toxic than the actual amount of berberine contained in it. The chemistry of Rasaut was therefore studied. It has been found that it contains (a) absolute alcohol insoluble portion which behaves like a saponin, (b) absolute alcohol soluble and acetone soluble which is berberine and (c) absolute alcohol soluble and acetone insoluble which is a degradation product of berberine. This degradation product is produced on heating. It gives tests for alkaloids but does not form salts with dilute mineral acids, is toxic to frogs and contains CHO group. The root of berberis contains besides berberine, small quantities of another alkaloid. The pharmacology of this alkaloid and the degradation product are under investigation.

Chaksu.

The pharmacological investigation of Chaksine sulphate was continued during the year. Symptoms produced in frogs, mice, rabbits and dogs have been studied and the following features were observed.

Depression of respiration, muscular twitchings and muscular paralysis were the common symptoms observed in all these animals. In frogs the skin on the dorsum showed a peculiar golden greenish hue which became quite marked when the animal was dead. Death was due to respiratory failure. Curiously enough, doses which are lethal to dogs during winter, had none or very little effect on these animals during summer.

The phenol coefficient was determined. The highest concentration in which *Chaksine* solution could be prepared was 1 in 400 in normal saline. This solution, when tried for the determination of its phenol coefficient, had no effect on the growth of B. Typhosus (24 hours culture in lemcobroth) when observed after 24 hours and 48 hours. The alkaloid is antipyretic in maximum tolerated doses. In small doses the effect is variable.

Further work on the pharmacology is being done.

The nelumbium alkaloid.

The crystalline sulphate of *Nelumbium Alkaloid* (the micro analysis of a sample sent by us was made in Germany before the war and was shown to be slightly impure) was prepared. It softens at 210°C and melts at 235°C. The constitution of the alkaloid is being studied.

UTTANGEN.

The chemistry of *Uttangen* was studied at length. It contains fixed oil, resins, colouring matter and two crystalline principles—one tasteless and the other a bitter substance.

BHAWAR.

The therapeutic uses of *Bhawar*, the chemistry of which was studied last year, are under investigation.

LANA.

Lana, which was shown to contain Vitamin C and tartarates, was tried. It has a feeble purgative action. It was tried by me and by another colleague and further trials will be given in hospital.

WITHANIA.

Milk clotting enzyme of *Withania* coagulans is being isolated and studied.

25. Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay.

THE ALKALOIDAL SUBSTANCE FROM PAPAYA SEEDS.

With the work reported in the last year's report the investigation on *Solanum xanthocarpum* was practically concluded. Full attention was directed afterwards to the chemical and pharmacological study of *carpasemine*, the "alkaloidal" substance isolated from *Papaya seeds*. Carpasemine on hydrolysis yielded a product, m.p. 149°C (vide last report). This product on oxidation gave a crystalline, colourless substance melting at 203–5°C. It is much less soluble in water than the parent substance and is different from the direct oxidation product of carpasemine reported last year. Various other properties of this hydrolysis product and of carpasemine have in addition been investigated during this year and the various substances obtained in the study have been sent for microanalysis, the results of which are awaited with interest. The results of the toxicity tests with carpasemine as well as its effects on the heart and blood vessels were reported last year.

Since *papaya seeds* are reputed to have an emmanagogue action, the action of carpasemine was studied on excised strips of uterus and intestine of rats and rabbits. High concentrations of the substance were found to inhibit the movement of the organs, but no such action was observed in intact anaesthetised or decerebrated dogs or cats. The action of carpasemine on other functions in higher animals was studied, but no marked action has been observed. Its action on unicellular organisms was also studied and it was found to have no effect on the growth of *B. coli* in cultures, as well as no effect on the movements of paramæcia. The *papaya seeds* are also said to be anthelmintic and hence the action of solutions of different strengths (1 : 20,000 to 1 : 1,000) of carpasemine, was studied on lower organisms like earthworms. It was observed, that in stronger solutions (1 : 3,000 to 1 : 1,000), their movements were slightly diminished but only some of the worms were found dead even after an exposure for 18 hours.

Similarly, its action on small fishes was studied and it was found that the fishes in stronger solutions (1:1000) were found dead within 3 hours while those in the weaker solutions were found living even after 24 hours. Further work is being done in this connection.

THE ALKALOIDAL SUBSTANCE FROM PAPAYA LEAF.

Carpaine, the alkaloid present in the papaya leaf, which has been already studied by Barger (Jour. Chem. Soc. 1910, *97*, 466) was isolated for comparative study. It was found that the pharmacological and chemical properties of carpasemine differ much from those of carpaine. The two substances are found to be quite distinct and do not appear to be even related to each other. The pharmacological investigation of carpasemine is now nearing completion, but it is proposed to study the pharmacology of some of its derivatives, when their nature is known and when they are prepared in sufficient quantities.

OTHER DRUGS UNDER INVESTIGATION.

The *Daemia extensa* leaves were found to contain a bitter substance perhaps a "glucoside" but its quantity seems to be too small for chemical isolation. Different extracts of the drug have, therefore, been prepared and their pharmacological study has been undertaken. Some preliminary experiments with the extracts are being performed. If a particular extract is found to have any pharmacological activity, it will be further studied ; and attempts will be made to isolate the active constituent of such an extract.

Work on *Erythrina indica* is being carried out, particularly that on the separation of the alkaloid, other than hypophorine. It is found that the crude preparations from the seeds, free from hypophorine, possess "curare-action".

Preliminary work, particularly chemical, on *Eclipta alba* is in progress.

26. Indigenous drugs enquiry under Dr. J. C. Gupta, Professor of Pharmacology, School of Tropical Medicine, Calcutta.

INDIGENOUS DRUGS.

Essential oils of some indigenous plants—The Rideal Walker coefficient values of the essential oils of *Ocimum basilicum*, *Ocimum sanctum* (green), *Ocimum sanctum* (red), *Curcuma aromaticus*, *Hemidesmus indicus*, *Pistacea integerrima* and *Homalomena aromatica* were studied last year; the results have been published in April, 1942, I. M. G. Vol. LXXVII, No. 4.

ALKALOIDS OF RAUWOLFIA SERPENTINA.

The extract, total alkaloids and *serpentine* show marked hypotensive properties, *ajmaline* and *serpentinine* on the contrary, are hypertensors. In experimental hypertension all of them induced hypotension, serpentine producing the maximum effect amongst them. *Serpentine* could be considered to be the chief pressure reducing factor amongst Rauwolfia alkaloids. *Ajmaline* and *serpentinine* stimulate, whereas *serpentine* depresses the intestine. The first two would account for the purgative effect of the extract reported by clinicians (I. J. M. R. Vol. XXX, No. 2).

Of the alkaloids isolated so far, *ajmaline* and *serpentine* stimulate the nervous system while *serpentinine* produces slight sedative action. The alcoholic extract is far more sedative and hypnotic than *serpentinine*. A method of extracting the total alkaloids avoiding any drastic treatment has been evolved.

Rauwolfia serpentina—Comparative pharmacology of the purified total alkaloids obtained from different regions—Bengal, Bihar, and Dehra Dun was studied. The total alkaloid obtained from the Dehra Dun sample caused a greater fall of blood pressure than the total alkaloid extracted from the Bihar sample but the fall was prolonged and more persistent with the Bihar *Rauwolfia serpentina* alkaloids. Total alkaloids of Bengal *Rauwolfia* produced intermediate results as far as hypotensive effect was concerned. Toxicity and the sedative effect of the Bihar and Bengal *Rauwolfia* total alkaloids were more than the total alkaloids of Dehra Dun *Rauwolfia*.

Other minor differences in the action of the three varieties of the total alkaloids of Bihar, Bengal and Dehra Dun on heart, uterus and intestines were noticed.

Vitex peduncularis and Vitex negundo—Effective antihaemolytic agents have been demonstrated in the extracts of *vitex peduncularis* leaves and *vitex negundo* root cortex. The preliminary work was done with a commercial preparation. Experiments are in progress to find the most suitable method of extracting the active principle which seems to be thermolabile.

Vitamin C content of some common summer fruits of Bengal—Vitamin C content of the following fruits have been estimated (i) *Carissa carandas*—6 mg per 100 g. (ii) *Spondias magnifera*—immature fruits—23 mg; mature fruits 56 mg per 100 g. (iii) *Flacourtia cataphracta*—less than 1 mg. per 100 g.

Indigenous mydriatic drugs—Samples of *Hyoscyamus niger*, *Atropa belladonna*, *Datura fastuosa* were collected from different sources in India and their alkaloidal contents determined.

Table

Name of plant	Parts used	Crop year	Total alkaloid B. P.	Total alkaloid (K. S.)	Scopalamine	Hyoscyamine
Atropa belladonna	Roots	1940	0.290	0.226	0.052	0.174
,,	,,	1941	0.510	0.488	0.063	0.425
,,	,,	1942	0.480	0.499	0.060	0.439
,,	,,	1942	0.480	0.515	0.073	0.442
,,	,,	1942	0.456	0.483	0.099	0.384
Datura fastuosa var. alba	Fruits	1942	..	0.223	0.192	0.031
,,	Roots	1942	..	0.240	0.151	0.089
,,	Seeds	..	..	0.300	0.248	0.052
,,	Leaves	1942	..	0.105	0.079	0.026
,,	Seeds	1942	..	0.337	0.254	0.083
,,	Leaves	1942	..	0.126	0.083	0.043
Datura fastuosa var. niger	Seeds	1942	..	0.383	0.288	0.095
,,	Leaves	1942	..	0.143	0.091	0.052

Anthracene purgatives—A method of standardization of such drugs as *aloes, rhubarb, ipomoea, cassia* by estimating the hydroxy methyl anthraquinone has been worked out : its universal adoption is recommended for obvious reasons. Samples of *Rheum* and *Cassia* have been investigated from this point.

Table

Rhubarb	Ash	Alcoholic solution extractive	Hydroxymethyl anthraquinone percentage
1. Sikkim L I ..	10.7	45.4	3.21
2. „ L. 2 ..	19.0	29.7	2.78
3. „ G 1 ..	17.5	20.4	2.45
4. „ G 2 ..	34.5	9.6	1.98
5. Chinese ..	16.0	41.6	2.64
6. European ..	8.5	36.9	2.38
7. Cassia fistula Bengal ..	..	..	0.86
8. C. sophora „ ..	..	..	1.10
9. C. tora „ ..	..	..	1.10

Insecticidal drugs—derris and pyrethrum—Samples of insecticidal plants such as pyrethrum and derris were investigated chemically.

Table

Source	Crop year	Grade	Pyrethrum percentage
1. Mayurbhanj	1941	Open	0.702
2. Tangmarg (k)	1940	Open	0.875
3. Baramula (k)	1940	Open	0.830
4. Badgam (k)	1941	Open	1.130
5. Borian (k)	1941	Open	1.234
6. Tangmarg (k)	1941	Open	1.150

Pharmacologically pyrethrum produces prolonged stimulation of respiration after an initial period of apnoea of short duration.

Daemia extensa—The preliminary work has been completed. It has a very powerful stimulating action on uterus *in situ* as well as on an isolated uterus. It seems to stimulate all simple muscle fibres and causes a persistent rise of blood pressure which may tend to fall below the initial level after about a quarter of an hour when given intravenously. This drug is being worked out with a view to its use as a substitute of *Ergot* preparations.

Vanda roxburghii—The preliminary work suggests it to have a sustained respiratory and circulatory stimulant action of a minor degree ; the stimulant action is more manifest under depressed conditions of respiration and circulation.

Berberis aristata—The action of *Umbellatin,* the alkaloid isolated from *Berberis aristata* against paramaecium has been investigated. It kills the flagellate only at high concentration (1 in 800).

Chemotherapeutic drugs.—The pharmacology of *Stilbene* a leishmanicidal agent in kala-azar has been investigated. It lowers blood pressure by directly acting on the arterioles and capillaries. The isolated mammalian heart in small doses is stimulated while the isolated intestines respond by slight increase of tonicity.

Reticulo-endothelial efficiency and chemotherapeutic drugs.—The reticulo-endothelial efficiency as measured by the congo-red clearance of plasma (called " Congo-red index ") was investigated in Rehsus monkeys on health, in experimental malaria and under quinine medication in health and disease. The action of *stilbene* on the reticulo-endothelial efficiency as measured by congo-red index was also investigated. The investigation is still continuing. Preliminary results suggest that quinine in health causes a slight increase of the reticulo-endothelial efficiency (less than 10%). In malaria the efficiency is lowered (30% or more). Quinine in malaria seemed to increase the efficiency specifically as the reticulo-endothelial efficiency returns to normal with the symptomatic cure of malaria.

Blood substitute—In collaboration with the All-India Institute of Hygiene, post-haemorrhagic shock preventing action of several serum protein preparations was tested on cats. The investigation is not yet complete. Preliminary work proves the efficacy of dried mammalian serum albumin in suitable concentration and properly salted in sustaining blood pressure after haemorrhage. The results obtained with heterologous serum proteins liquid or dried are not yet sufficiently encouraging.

27. Indigenous drugs enquiry under Bt.-Col. Sir Ram Nath Chopra, C. I. E., I. M. S. (R) at the Drug Research Laboratory, Jammu, Kashmir.

Indigenous drugs.

Medicinal plants—The term 'digitalin' has been employed to different products and does not convey anything definite. The isolation of active glucosides of *Digitalis purpurea* and *D. lanata*, which are being grown in Kashmir on a large scale, has been taken up in order to prepare a uniform, stable and standardized product for therapeutic use. One fraction of the glucosides has been prepared and biologically assayed, and has been found to be quite potent. Further work is in progress.

In these days of acute shortage of quinine, various reputed indigenous antimalarial remedies are being carefully investigated and clinically tested. The two drugs *Tinospora cordifolia* and *Aconitum heterophyllum* are widely used in this country and are under investigation at present. Preliminary reports in this connection are encouraging.

Work on the seasonal variations in the control of active principles, the altitude and localities at which the maximum yield is obtained and the best time for collection of the parts used in medicine is being carried in connection with the following plants :—

Belladona, Pyrethrum, Podophyllum, Digitalis and Ephedra.

28. Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

Investigations during the year 1942 were concerned with the pharmacology of new synthetic sulphonamide drugs with special reference to their antimalarial activity and the problems of immunity in monkey malaria.

Synthetic anti-malarial drugs.

Two years ago it was reported that sulphathiazole had a curative effect in *knowlesi* infection in the rhesus monkey. Since then a large number of drugs of sulphonamide group have been synthesized in the Haffkine Institute, and several of them have been tested for their antimalarial activity in the rhesus monkey. Of these, two drugs, viz. $2N^1$-Sulphanilamido-5-ethylthiazole and N^1-Methyl sulphathiazole were found to possess an antimalarial activity. The former drug —the ethyl derivative—given orally in a single dose of 1 g., caused disappearance of the parasites in a moderately severe infection (100 parasites per 10,000 normal R. B. Cs.). Treatment with 1 g. doses given daily for three consecutive days produced a radical cure. The drug, therefore, was found to possess as good an action in monkey malaria as sulphathiazole. Its pharmacological action was, therefore, investigated in detail. It was found that the drug was quickly absorbed from the gastro-intestinal tract and was chiefly excreted through the kidneys. Its toxicity both acute and chronic was low. It produced very little action on the cardio-vascular, genito-urinary, gastro-intestinal and respiratory systems. Very high doses of the order of 20 to 40 mg. per kg. when injected intravenously produced a slight depression of the heart ; the blood pressure and the plain muscles in the body were also slightly depressed.

The other drug—the methyl derivative—was found to be less efficacious in its antimalarial activity. Investigations of its toxicity showed that it was more toxic than the ethyl derivative. Its absorption and excretion was about the same as the other drug.

Immunity in monkey malaria.

(a) *Effect of spleen extracts*—Extracts of spleens removed from ' immune ' monkeys have been tried previously by some workers to see whether they could confer any immunity to normal monkeys when administered parenterally. Such extracts, however, proved to be too toxic to be administered in adequate doses. Tissue extracts generally contain a variety of substances like cholines and amines which are very active pharmacologically and produce marked toxic reactions in very small doses. These toxic substances were, therefore, removed from the extracts. Sensitive physiological tests were employed to ascertain the absence of these toxic substances. After their removal it was found that adequate doses could be administered to normal monkeys without producing any severe toxic reactions. Control experiments were made with spleen removed from normal monkeys to see whether extracts of such spleens produced any immunity when injected into normal monkeys. It was found that such extracts did not give any immunity. Extracts of spleens removed from ' immune ' monkeys were then tested similarly and it was found that such extracts conferred a certain degree of immunity to normal monkeys. These studies are now in progress on a quantitative basis.

(b) *The rôle of cellular and humoral factors*—It was reported last year that even when 80% of the ' immune ' blood was removed from an ' immune '

monkey and replaced by normal blood, the monkey still retained its immunity towards *knowlesi* infection. This showed that even when the humoral factor was removed to a considerable extent, the immunity was not demonstrably affected. The cellular factor evidently was chiefly responsible in retaining immunity in such experiments. The rôle of this 'cellular' factor was, therefore, investigated further. It is now generally known that when a large portion of these cells is removed by removing the spleen, the animals get a relapse and when this relapse is treated adequately the animals again pass into a chronic stage and are immune to further super-infection. When such immunity was established in splenectomized monkeys, attempts were made to break it by blocking their reticulo-endothelial cells by giving intravenous injections of India ink. It was found during these experiments that the ordinary commercial India ink was not suitable for this purpose because adequate doses of the ink could not be given with safety. The ink—or suspension of pure carbon—was, therefore, prepared in the laboratory and standardized by the biological method described two years ago. In all experiments this standard ink was used for blockade. When adequate doses of the ink were administered to splenectomized immune animals their blood was replaced by normal non-immune blood by the cross-circulation method. It was found that if 70% or more of such replacement was effected, the animals got a relapse and succumbed to the infection. Thus replacement of immune blood by normal blood in animals with their reticulo-endothelial cells blocked was shown to break the immunity.

MATERNAL MORTALITY.

29. Haematological unit under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta.

During the period under review, the work on the above enquiry was carried on along broad lines, indicated in last year's report with such alterations in details as were required by the exigencies of time and circumstances.

Animal experiments.

Last year, the effects of sulphapyridine on the haemopoietic system of Rhesus monkeys, living on the ordinary stock diet of gram, green vegetables and banana were studied. How far these effects are controlled by the nature of diet fed to animals, formed the subject of this year's investigation. For this purpose, a low protein—high fat diet, consisting of rice, codliver oil and olive oil, mixed with a special salt mixture was used and 5 monkeys were experimented on. Of these animals, one served as a control and the remaining 4 were fed this diet for a month. After they had got used to it, sulphapyridine in watery suspension was administered to them in varying doses, the animals being maintained on the same artificial diet both during the period of drug administration as well as for a month after it. One monkey died during the course of these experiments but the remaining 3 survived the whole experimental regime.

Weekly blood counts were made on all these animals throughout the period of observation. The data obtained are interesting so far as they go, but then they do not seem to go very far ; for while they clearly show that the deficient diet as contrasted with the stock one had a definite retarding effect on the recovery from the anaemia, mild or moderate, caused by sulphapyridine, they do not throw much light on the interaction between sulphapyridine and deficient diet in their cumulative effect on the haemopoietic system of monkeys. But as the number of animals used is too small to admit of any definite conclusions, work on this line is still being continued.

Further animal experiments, made during the period, had in view quite a different object—that of elucidating the nature of anaemia, resulting from chronic malarial infection. Experiments on this line were therefore started by inoculating 10 monkeys with *Plasmodium knowlesi*, the dose used in inoculation, varying in individual cases from 3×10^8 to 2×10^8 parasites. Within a week, all the animals were infected. Quinine injections had to be given to all but one, to avert fatal termination ; the dose and time of administration of these injections, however, had to be so arranged as to produce, not a complete cure but a state of chronic infection in the animals. During the whole period of observation, blood counts were made at weekly intervals, or oftener if necessary, and blood films were examined almost daily for malarial parasite after the infection had taken.

It was quite obvious from the beginning of these experiments that nothing was easier than to produce anaemia ; often of a marked degree, in these animals by this method. And since the occurrence of anaemia and infection were more or less coincident and since anaemia always persisted even after quinine injections, it appeared profitable to analyse more closely 113 blood counts made on these animals in the post-infection stage in an attempt to get some insight into the nature of anaemia produced, as a result of infection. Previous work under the

enquiry had shown that normal M. C. V and M. C. H. figures for the Rhesus monkeys were $74.2 \pm 7.57 \mu^3$ and $22.1 \pm 2..58$ YY ; from which, by applying twice the standard deviation on either side the mean, the extreme values for these haematological constants are easily calculated to be 89.34 – 59.06 cubic micra and 27.26 – 16.94 micromicrograms respectively. Accepting these as the normal ranges, and applying them to 113 counts mentioned above, it was found that so far as M. C. V. values were concerned, 82 of the counts fell within normal range, 29 beyond the maxim and 2 below the minim values ; and so far as M. C. H. values were concerned, 99, again fell within the normal range, 10 beyomd the maxim and 4 below the minim values. As for the counts outside the normal range, it was further noted that in not a single monkey these constituted the major portion of the total counts made during its anaemic stage, so that macrocytosis and microcytosis are to be regarded more as tendencies in, rather than types of, anaemia under discussion. It can therefore be concluded, tentatively at any rate, that the anaemia due to chronic malarial infection, produced under experimental conditions is predominantly of a normocytic type. It may be added, *en passant*, that this remarkable tendency towards macrocytosis manifested itself whenever there was a sudden precipitous drop in the blood levels with sharp reticulocyte crisis, and these, in their turn were seen to occur whenever there was a sudden parasitic relapse in the course of a chronic infection.

As regards the other line of work on the haemolytic anaemia in monkeys due to specific anti-monkey haemolysin, suffice it to say, that a paper entitled " Studies on Haemolysis " embodying the results of the experiments on the line is very soon going to be published in the Indian Journal of Medical Research.

Studies on normal and abnormal blood conditions in human beings.

Pari Passu with these animal experimentations studies were continued as usual on the normal and abnormal blood conditions in human beings.

Normal blood conditions—Work was mainly confined to the accumulation of further data on the haematological standards of people ordinarily resident at high altitudes such as Gangtok and Kalimpong, both situated on the Himalayas within 4000 to 6000 ft. above sea-level. At Gangtok, complete blood counts were done in a sample of 107 adults of either sex, drawn entirely from hill tribes ; at Kalimpong such examination was made in a series of 115 persons of which 44 were Europeans and Anglo-Indians, while the rest belonged to different hill tribes. In addition, in Kalimpong, simple haemoglobin determination from finger blood was done in a mixed group of 158 individuals. Determination of blood grouping and sedimentation rate formed a feature in many of the cases.

Abnormal blood conditions.—Anaemia, especially that of the macrocytic variety occupied the centre of the field. As stated in last year's report there were always under the Anaemia Department of the School some 10 to 12 cases of this anaemia in the hospital, available for investigation throughout the year and as in previous years new preparations of liver were frequently tried on these patients in an attempt to find the fraction of liver which is most suitable in these cases.

An innovation which deserves special mention this year is the establishment of a Civil and Hospital Transfusion service in the department. With the establishment of this service, not only has the work on blood grouping and blood transfusion received a fresh impetus, but the whole outlook on the work has acquired a new orientation because of its wide social significance at the present juncture.

Since January, 217 complete blood counts on monkeys, 222 on ' normals ', living at a high altitude, and more than 300, on anaemic patients have been done under the auspices of this enquiry.

The haematological unit forms a part of the haematological research department of the school and is under the control of the haematological research worker. The work of the unit is therefore dependent to a certain extent on the general research work of the haematological research department.

30. Study of the blood picture in normal pregnancy under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta.

This enquiry is being undertaken in collaboration with Dr. R. B. Lal, Professor of Vital Statistics and Epidemiology of this Institute and Dr. S. Mitra, Honorary Obstetrical Surgeon to the Chittaranjan Seva Sadan and Carmichael Medical College Hospital, Calcutta.

The enquiry has as its object the definition of the physiological blood picture in its haematological and biochemical aspects, in Indian women during pregnancy and the current report covers the third and the last year of the study.

During the year, cases were drawn from several prenatal clinics conducted under the auspices of the Bengal Red Cross Society and from cases attending the outdoor of the Eden Hospital. The Institute's Maternity and Child Welfare centre was closed in April due to the exodus from Calcutta.

Altogether 445 bloods were examined haematologically for the first time and in 228 cases, biochemical examinations were also made ; 14 'repeat' and 7 postnatal examinations were made. This brings the total number of bloods examined haematologically to 1,552.

Of these 445 specimens, supplementary data to complete the picture were available in 250 instances and in 173 cases the woman was judged clinically normal by the standard set up. The number of such normal cases now totals 423, while a further group of 250 bloods fall into the class " near normal ". In 159 cases, except that stool examination was not possible, no abnormality could be detected in the woman.

These and other groups associated with the presence of one or more abnormalities will be submitted to examination and analysis in the early part of 1943.

The distribution by months of pregnancy of these three groups of cases is as follows :

	TOTAL	-3m.	3m.	4m.	5m.	6m.	7m.	8m	9m.
Clinically normal and complete as regards data.	423	28	13	39	91	42	67	57	86
Near normal, complete as regards data.	250	19	19	20	23	37	44	40	48
Clinically normal lacking only stool examination.	159	7	15	12	20	32	31	14	28

The Price Jones curve was traced in 191 cases of bloods falling into the normal group.

In 5 cases gastric analysis was done in addition to the usual biochemical tests.

Collection of specimens will close in February, 1943.

31. Enquiry into the maturity range of infants under Dr. L. S. Ghosh at the All-India Institute of Hygiene and Public Health, Calcutta.

This inquiry has as its object the mapping out, as far as may be practicable, of the data and measurements delimiting the premature (viable) Indian infant from the mature infant, in the hope that standards may emerge which can be applied by the agent of delivery in community maternity schemes. Clear diagnosis of prematurity may be expected to lead to measures for the protection of premature infants, and so effect a fall in infant mortality.

The recent records of deliveries conducted at the Eden Hospital and Chittaranjan Seva Sadan were selected to secure the data and measurements required. It was decided to record at least 4,000 consecutive cases, 2000 from each hospital, 1000 reclassified premature infants and 1000 reclassified mature infants in each instance.

A panel was formed consisting of two leading consultant obstetricians, a statistician, the officer-in-charge of the inquiry and the obstetrician subsequently appointed to record the data. This panel drew up a key to the reclassification, upon clinical data only, of the single fresh born infant in regard to maturity. This key was then submitted to the recording obstetrician so that uniformity of diagnosis might be effected in selecting the cases in order to get the distribution required.

A schedule was drawn up subsequently and transference of data was undertaken from the 1st July. Up-to-date, 3,800 schedules have been completed and the required number will be completed by the 15th December.

Analysis will commence at the beginning of 1943.

OTHER RESEARCHES.

32. Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

The work done by the enquiry may be considered under two heads—nutrition and microbiology.

Nutrition.

Work was continued on the problem of utilisation of proteins and their degradation products, by heart fibroblasts of the embryo chick cultivated in vitro, by the hanging-drop technique. The higher fractions of protein hydrolysis e.g. the proteoses and a preparation " substance X " of Willmer and Kendal produced satisfactory growths. The utilisation of the proteoses however was dependant on the presence of plasma, whereas " substance X " produced abundant growths even in the absence of plasma in the medium. In the latter series though the growth was abundant the appearance of the individual cells tended to indicate that " substance X " apart from a definite stimulating effect on cell division and growth, failed to satisfy fully, the nutritive requirements of the fibroblasts, and the picture could be further improved by the addition of plasma to the medium. It was thought that the estimation of the amount of nitrogen added to the culture media and of that recovered from it after the cultivation period would furnish information regarding this point. A series of experiments involving microchemical analysis were undertaken. Both micro-Kjeldhal and colorimetric methods were given a fair trial. The amount of material available for the purpose being very small the results obtained from such infinitesimal quantities were not found to be uniform even after several attempts. These were therefore not taken into consideration. It was then decided to commence another series of these cultures using the carrel flask technique, where more material was available for the tests. Several preliminary experiments were carried out and cultures were put up in Carrel D 3.5 flasks. Explants were taken from the heart of the embryo chick of 7-10 days incubation. The media consisted of tyrode solution, embryo extract and fowl plasma. Phenol red was used as an indicator. The explant was first embedded in the plasma clot which formed the solid phase, the liquid phase composed of tyrode solution, embryo extract and phenol red was added subsequently. The flasks were sealed and incubated at 37°C. At the end of 48 hours of incubation, the liquid phase was renewed after washing the cultures with tyrode solution. Thereafter the liquid phase was renewed whenever it was considered necessary. The cultures were examined from time to time and the amount of growth noted. The cultures could thus be maintained in an actively growing state for much longer periods than was possible with the hanging drop technique. Further study of proteoses and " substance X " of Willmer and Kendal were however discontinued for the time being, owing to the increasing difficulty in obtaining supplies of Witte's peptone, from which these substances are prepared by fractionation.

Microbiology.

The work under this head may be subdivided into three sub-heads (a) investigation of the aetiological factor of trachoma, (b) assisting the blood Bank in its work and (c) teaching facilities to the D. P. H. students.

Investigation into the aetiology of trachoma—Trachoma is a highly communicable disease. In India it claims millions of victims and causes blindness

and disability in a high percentage of them. In Bengal, particularly in the rural areas its incidence is not inconsiderable. Considering its public health importance relatively little work has so far been undertaken in India regarding its aetiological factor. At present there are two views regarding its infective agent. Majority of workers agree that it is caused by a virus, while the second school maintains that it is a rickettsial infection, the body louse acting as the intermediary host. Due consideration is being given to both these views. The Tissue Culture Enquiry started its investigation at the beginning of the current financial year with the cooperation of Col. E. O'G. Kirwan, head of the Eye Infirmary, Medical College Hospitals, Calcutta. The enquiry has been able to investigate several carefully selected cases. Scrapings were taken from the upper palpebral conjunctiva of early and untreated cases, after thoroughly washing the conjunctival sac with sterile normal saline. The use of antiseptics and local anaesthetics was avoided as these are known to have a deleterious effect on the infective agent. Smears were made from the material on glass slides for histological examination after staining, and inoculation experiments were also carried out in M. rhesus monkeys. In some of the cases examined Halberstaedter-Prowazek inclusion bodies typical of trachoma were demonstrated and microphotographs of the same were taken. Further work is proceeding.

Blood Bank work—The services of the experimental cytologist were utilised by the Microbiology section for Blood Bank work between June and October, 1942.

Teaching facilities to the D. P. H. students—In the absence of any other arrangement the tissue culture enquiry has been arranging demonstrations and lectures on virus diseases for which tissue culture methods are indispensable. In this connection the various methods of tissue cultivation and cultivation of the agent of some of the more important virus diseases as well as the histological appearance of the lesions were demonstrated.

33. Enquiry into medical mycology under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta.

Fungicidal and fungistatic properties of a combination of the three dyes acriflavin, brilliant green and gentian violet.

The work on the fungicidal and fungistatic properties of a combination of the three dyes acriflavin, brilliant green and gentian violet was completed. A solution of these three dyes was tried on some ringworm fungi *in vitro* and also clinically in certain septic conditions of the skin. Both these experiments proved successful.

As a fungistatic, the solution when mixed with the culture media inhibited the growth of the common ringworm fungi and the staphylococcus in 1/100,000 dilution. As a fungicidal agent, the solution proved effective in killing them when brought in contact for 1 minute in 1/2000 dilution, and in 1/4000 dilution if the contact was for 10 minutes, and so on.

Clinically the solution has given very satisfactory results in the treatment of staphylococcus infection of the skin in general, and also of sycosis barbae and pustular folliculitis both of coccal and fungal in origin. This solution has been found to possess better therapeutic value and being less irritating is better tolerated than those hitherto tried.

Another great advantage gained by this experiment is the saving of these costly dyes difficult to obtain at present, and also the economy in the cost of treatment. The table below shows the quantity of the dyes required to make up 100 c.c. of the solution which were in use before and that required for the triple dye solution :

(1)	Solution of gentian violet 4%	– 4 grammes	for 100 c.c. of the solution.
(2)	Solution of gentian violet and brilliant green–		
	Gentian violet	– 2 grammes	" " " "
	brilliant green	– 1 gramme	
	Total	– 3.0 grammes	
(3)	Solution of triple dye		
	Acriflavin	– 0.1 gramme	
	Brilliant green	– 0.25 gramme	" " " "
	Gentian violet	– 0.25 gramme	
	Total	– 0.6 gramme	

It is clear from the above table that the quantity of the dyes has been reduced to 1/5th of that used before, and this is certainly a great advance, especially at the present crisis when the dyes are very difficult to obtain and the cost has gone up to five or ten times.

Along with this experiment " Dettol " a proprietary antiseptic was put to test. Dettol is largely used at present by the medical practitioners and also in the hospitals. This work has also been completed and the results are being analysed.

The study of the immunity of the ringworm fungus (epidermophyton floccosum) in the laboratory animals.

The first part of the work has been completed and it has been found that (a) ringworm infection can be produced artificially in young monkeys, (b) after the first attack the monkeys remain immune to further infection for at least one year and (c) serum of the immune monkeys can give immunity to other monkeys.

The second part of the proposed work—experiments with fungi causing hair ringworm and the cross immunity with the fungi causing body ringworm, and also experiments to produce immune serum for human use could not be taken up in the present year.

The serological reaction (complement fixation test) in cases of seborrhoeic dermatitis.

The exact actiology of seborrhoeic dermatitis still remains unknown – and a large number of cases of generalised dermatitis require to be properly classified. Seborrhoea is said to be one of the common causes of generalised dermatitis without any positive evidence to justify the diagnosis. Some workers are of opinion that both seborrhoea and seborrhoeic dermatitis are due to the

infection with the fungus *Malassezia ovale.* The same organism has been said to be the cause of acne.

The present investigation has been undertaken to determine whether a diagnostic criterion could be established by a serological reaction with the antigen prepared from the culture of the fungus *Malassezia ovale.* About 100 cases have been studied and the results are very satisfactory. About 70 per of these clinically seborrhoeic cases have given a definitely positive serological reaction, the control cases remaining negative. There are still some difficulties to overcome. Firstly *Malassezia ovale* is difficult to culture and several strains are required to prepare a good antigen. Secondly standardisation of the antigen has not been perfected as yet to give uniformly satisfactory results in all cases. These factors are delaying the progress of the work.

To determine the oxidase contents of oil Psoralea corylifolia and other allied oils and the rôle of the oxidase in the production of melanin.

Incidence of leucoderma is very common in this country and the disease though harmless carries great public sentiment with it. The treatment of this disease has a great public demand in this country. There is at present no specific treatment giving satisfactory results.

The oil expressed from the seeds of *Psoralea corylifolia* seems to have some property of restoring the pigment melanin when rubbed in the patches. Local injections of the same oil in the patches hasten the process to a certain extent. But this method of treatment falls far short of expectation.

It is not known what part of this oil plays the part in pigment formation. It is said that 3:4 dihydroxyphenylalanin (Dopa) is oxidised into melanin and an enzyme of the nature of oxidase is responsible for the conversion.

Following this analogy the present investigation has been started. The oxidase from the oil expressed from the seeds of *Psoralea corylifolia* and *Moringa pterygosperma* are being isolated and the clinical values of these are being ascertained. Other contents of the oil likely to have any effect in pigment production are also being tried. So far the oxidase alone does not seem to play any inportant part in producing melanin.

Treatment of alopecia areata.

Alopecia areata—or the sudden patchy loss of hair is a disfiguring condition. Though the prognosis is invariably good still it causes great anxiety and nervousness in patients who do not respond to treatment even for months. In order to shorten the period of suffering, injections in the affected areas with some local irritants were tried. The results justified this experiment, and up to the present, about 58 cases have been treated by various stimulants with invariably good results.

Treatment of leucoderma.

Treatment of leucoderma which has assumed considerable importance in this country is still being carried out with the application and local injections of the oil *Psoralea corylifolia.* A large number is receiving treatment and a good many of these have been cured.

Attempts are being made to improve the method and technique of this treatment.

34. Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta.

The work carried out during the year 1942 may be dealt with under the following heads :—

Field work.

Surveys in the following areas were carried out mainly with a view to ascertaining the species of filarial parasites prevalent in them :—

Damda village in Drug district (Central Provinces) ;
Bassein in Thana district (Bombay Presidency) ;
Muzaffarpur (Muzaffarpur district) ;
Khagria (Monghyr district) in Bihar ; and
Ramnagar (Benares State).

In Damda, the infections were entirely *Wuchereria malayi*. In this village there were 80 cases of elephantiasis of limbs in a total population of 3,628. There was no case of elephantiasis of the genitals or hydrocele or chyluria in this area. The filarial infection rate (microfilarial-rate) was 20% (28 out of 140 apparently normal persons were positive for microfilariae). In many respects Damda is very similar to Ratanpur in Bilaspur district. It was once the headquarters of ancient Hindu rulers. It is situated 21 miles to the north of Drug, the headquarters of the district. The water supply is from tanks and ponds. Agriculture is the main occupation of the people who are mostly Hindus. There are no roads, no conservancy and no drainage.

A mosquito survey of this area was also made and the following species among others, were found to be prevalent in the area. The species were kindly identified by Dr. M. O. T. Iyengar. *Mansonioides annulifera*; *Anopheles culicifacies* and *Culex fatigans* (few).

In Bassien, infection with *W. malayi* was absent although one tank situated in the centre of the town was full of *Pistia stratiotes*. In this locality the infection and diseases were due to *W. bancrofti*.

At Muzaffarpur and Khagria, a total of 86 and 27 persons respectively were examined for microfilariae. The infection rates were 16% and 22% respectively. The infection in both the areas was found to be *W. bancrofti*.

In Ramnagar (Benares State) as in Benares city the infection was solely *W. bancrofti*. *Pistia* was found to be completely absent in the area and *Mansonioides* mosquitoes were not found.

It may be mentioned that in the course of a guinea worm survey in Jammu and that of the common endemic diseases in the valley of Kashmir carried out during this period, a search for filarial infection in both the areas was made, and it was found that the infection was completely absent in both these places in that State. *Culicine* mosquitoes and especially *Culex fatigans* were present in large numbers during the months of June and July in all the areas of the state excepting those at higher elevations. Dissections of wild mosquitoes caught from habitations from different localities both in the valley and in Jammu district were carried out for filarial infection, but there was no evidence of its presence in any of the areas examined.

Identification of adult filarial parasites.

Adult filarial parasites collected by Dr. Thomas Clive Backhouse at Rabaul from human cases in New Guinea and Papua sometime ago and sent to us by Prof. G. M. Heydon of Sydney reached us early this year. Included with them were also adult worms collected by Dr. Richards from Townsville. A careful examination of the specimens showed that they were *Wuchereria bancrofti.*

Specimens of adult worms recovered from the Nepalese troops stationed in Dehra Dun sometime ago and sent to us by Capt. John H. Bowie, I.M.S., were also found to be *W. bancrofti.*

Periodicity of microfilariae.

In order to study the phenomena of nocturnal periodicity of microfilariae, transfusion experiments were carried out. Blood from infected patients was drawn at night and transfused into volunteers who were found free from infection by repeated examinations of peripheral blood previously. Hydrocele fluids with microfilariae were injected into hydroceles known to be free from microfilariae. In the case of one blood transfusion, the recipient did not show any microfilariae in the peripheral blood either immediately after transfusion or subsequently. Out of five cases of hydrocele which received the transfused hydrocele fluid with microfilariae only two patients were available for continued observations for three months. One of them showed complete disappearance of the hydrocele through absorption of both the original and the injected fluid, while the other patient showed the presence of the embryos in the hydrocele fluid up to a period of 14 days. A two-hourly count of embryos in the hydrocele fluids made in the case of both the donor and the recipient did not show any great variation in their number.

Work on filarial allergen.

Serum collected from rabbits which were injected with guinea worm blister fluid, gave a strongly positive flocculation reaction with hydrocele fluid in all dilutions. Oedema fluids collected from non-filarial patients did not show any flocculation with the above serum nor with the sera of rabbits injected with hydrocele fluids. This work is in progress.

The treatment of allergy in filarial patients by the administration of filarial allergen which was continued this year, has given very satisfactory results.

Treatment of filarial infection.

The following drugs were tried in filarial infection for their filaricidal properties :—

Fantorin (Glaxo) ; Anthiomaline (May & Baker) ; 'M & B 744' ; and Antiphilarin tablets.

Fantorin is an organic trivalent antimony compound made by Glaxo Laboratories and consists of a 6.3% sterile solution in water of penta-sodium-antimony-bismuth catechol 2 : 4-disulphonate. It contains 8.6 mgs. of antimony per c.c. It is a product of low toxicity, is well tolerated and is administered intramuscularly or intravenously.

Anthiomaline is lithium antimony thiomalate made by Messrs May & Baker, London. It is an organic trivalent compound containing 16% of antimony. This is also a compound of low toxicity and is injected by the intramuscular route.

Both the above compounds have been tried extensively and appear to give good results in the infection and in the relief of diseases. This work is being continued.

'M & B 744' or diamidino-stilbene or 4 : 4 diamidino-diphenyl ethylene is an aromatic compound of the amidine group prepared by Dr. A. J. Ewins of Messrs May & Baker, London. This compound has been reported upon by Dr. L. E. Napier as effective in the treatment of kala-azar. It happened that two kala-azar patients who also had filarial infection at the time of treatment with this drug appeared to have overcome the latter infection also. A thorough trial given on two filarial patients subsequently did not yield any beneficial results.

Antipholarin tablets made by a local chemist contain Ammon. picras, Quin. flour, Arsenic trioxidum and Ext. thyroid. A full course of these pills was given to a total of 172 patients some of them showing the filarial infection and others with different types of the disease. This combination of drugs did not show any lethal effect on the parasite.

35. Enquiry into filariasis under Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Vizagapatam.

The history of the parasite *consispiculam guindiensis* has been studied. The natural history of the primary host *calotes versicolor* including its natural habitat, generic and specific characteristics, differences from closely related species of *C. maria* has also been studied. The important relation between the breeding season and the rise of infection rate has been brought out.

Life history.

A study of the developmental forms of *consispiculam guindiensis* in *calotes versicolor* has been made after experimental inoculation subcutaneously into lizards previously examined for absence of infection. The lizards were killed at specified intervals and the developmental forms of the parasite were recovered by dissection. For each stage, the site of injection, the site of recovery, time after injection, were all recorded and photographs obtained. The cycle is as follows. From the stage of infective larvae to the adult stage about 10 developmental forms are found. The infective larvae inoculated into the limbs migrate into the pelvic cellular tissue and thence into the mesentery which seems to be the final goal of the majority of the larvae introduced. By about the 4th day the oesophageal demarcation is complete and the rudiments of reproductive organs appear. The development of male genital organs appears late as compared with the female where differentiation is probable even as early as the 12th day. By about the 21st day sexes are well differentiated. Microfilariae never appear in the blood before the 45th day but commonly occur about the 72nd day. The time of maturation of the female worm averages about 64 days. Effects of hyperfilariation of the lizards are recorded. Histological examination of the sections of the mesentery showed reaction round the parent worm and the microfilariae. The mesentery was slightly thickened and some sections showed an increase in the celluar content. In the lungs, multinucleated eosinophilic giant cells were seen in the cellular exudate around the worm. The vessels and the alveoli in the lungs showed numerous microfilariae. On the

surface of muscle, around the microfilariae there were inflammatory changes with a fibrinous exudate and cellular reaction. The heart, colon, mesentery and other viscera were also studied.

36. Enquiries on filterable viruses and influenza virus under the Director, King Institute, Guindy, Madras.

Vaccinia virus.

The experiments initiated in 1940 to ascertain the relative value of chick membrane vaccine and the routine calf lymph in the prevention of smallpox were continued.

The field results for the second year of the experiment were analysed and a report forwarded to the Public Health Commissioner with the Government of India. The same technique was used as in the previous year and the chick membrane virus was suspended in tyrode solution as before. Following the results of observations on the keeping property of the virus in tyrode solution and 50% glycerine-tyrode solution, it is proposed to use the vaccine suspended in 50% tyrode-glycerine in future work.

In 711 cases of primary vaccination with chick membrane vaccine, a case success rate of 90.9% and insertion success rate of 70.7% were obtained against 89.2% and 73.2% in the previous year. In 362 revaccinations, the success rate was 12.7 (insertion 7.5%) as against 11.8 and 8.3 per cent, in the previous year.

Keeping quality.

The investigation on the keeping quality of the chick membrane lymph was continued. In 11 batches stored at -5°C. the potency was almost unimpaired at the end of a month, and in three for nearly three months. Four batches kept in tyrode and 50% glycerine-tyrode, showed no appreciable difference between the two suspending fluids in their keeping quality.

In Polal, a village in the experimental group, there were 11 cases of smallpox with one death during July and August. Six of these were among those vaccinated in childhood (several years previously) with the calf lymph and the 7th in a case who had been revaccinated with calf lymph 9 days previous to the attack. Two others had been vaccinated, also with calf lypmh 3 days previously. There was no attack among the chick membrane group.

Influenza virus.

No large series of cases were available for investigation. Attempts to isolate the virus from the throat washing of a few sporadic cases, clinically diagnosed as influenza were unsuccessful. In all cases, serial passage was continued up to at least 5 transfers, before the attempt was given up as unsuccessful. Further attempts will be made as cases become available.

Miscellaneous.

(a) *Poliomyelitis*—The spinal cord of a fatal case of poliomyelitis became available in August and attempts were made to establish a strain of the virus by passage in monkeys. Four monkeys used in the experiment escaped infection and no virus could be obtained.

(b) A case of *postvaccinal encephalitis* was investigated in September. Blood serum and cerebro spinal fluid were tested for the presence of the virus of vaccinia and their antibodies titrated. There was no evidence of the presence of the virus in either serum or cerebro spinal fluid taken 12 days after vaccination. Viricidal antibodies titration showed that the serum was active in 1 in 40 dilution (and to a slight extent 1 in 100), but there were no demonstrable antibodies in the cerebro spinal fluids.

Infective hepatitis.

Following an investigation of an outbreak of jaundice in Ootacámund in July affecting mainly the inmates of a residential school, the Health Officers of other municipal towns in the Province were circularised to report if there had been recent increased prevalence of jaundice in their areas. Many of the officers in the Southern districts reported that there had been such an increase but at the time the incidence had fallen. Four of these places where some cases were still said to be available for investigation were visited early in August. Seventeen cases of jaundice were seen, but most of them had more or less completely recovered. It was only in one institution in Tanjore that the sequence of cases suggested that the cases might be due to infective hepatitis. Even there, no active cases were available for investigation. Specimens of blood were obtained from 8 cases to see if a retrospective diagnosis could be made. Blood smears showed no significant abnormality. No parasites were seen. Agglutination tests against the Salmonella group of organisms were negative. Sera from some of these cases have been saved for further tests.

Towards the end of the period under report, a small series of cases of infective hepatitis became available at the Government General Hospital, Madras. In attempts made to isolate the virus from these cases, an agent capable of serially producing a characteristic lesion on the chorio-allantoic membrane of chick embryos has been recovered from 4 of these cases. Further work is in progress.

37. Kala-azar enquiry under the Director, Pasteur Institute, Shillong.

The outstanding experiment conducted by the enquiry during the year was the successful transmission of kala-azar to human beings by the bite of the infected sandfly *Phlebotomus argentipes*, Ann., and Brun. A 100% infection in human volunteers was shown to take place, thus solving a long-standing problem which had eluded workers in tropical medicine.

Experiments with human volunteers.

Five volunteers, all residents of the non-endemic area of Shillong and young adults, were made use of for these experiments. They had never left their homes in the non-endemic area of the Khasi Hills before the commencement of the experiments, and were subjected to a thorough examination before the experiments started including laboratory investigation and X-Ray examination of the chest. The following table gives the total number of flies fed on each together with the number of flies in each series found positive by subsequent dissection.

Table I

Names of the volunteers	Total flies fed	Total number of flies found positive after the feed	Time in days of confirmation of the infection after the bite of the first infected fly
U Wellington Masar	387	44	134 days
U Sohon Dome	401	26	223 „
U Hili Chalam	315	30	153 „
U Saran Lalo	384	42	140 „
U Bin Shullai	372	28	195 „

All the five volunteers showed clinical signs and symptoms of kala-azar. The clinical diagnosis was confirmed in each case by positive findings of *Leishmania donovani* both in smears as well as in cultures of spleen puncture material. All the five volunteers reacted well to antimony treatment. Between the first infective bite from the fly and the onset of symptoms (period of incubation), the shortest period was 134 days and the longest 223 days, the average being 24 weeks.

Experiments to determine the minimum number of bites from infected flies required to bring about an infection.

In this experiment mice were used as experimental animals. Only two mice could be used in this series as most of the case infected flies were made use of for human volunteers. One had bites from a single fly, and the other had four bites from infected flies. The former died after 4½ months and the latter after 2 months. Neither showed any signs of infection, e.g. enlargement of spleen or liver. No cultural examination was however made.

Studies on the bionomics of sandflies.

These studies were commenced only after the transmission experiments were successful. During the short period that has followed some observations on the bionomics of the fly were made and are given below.

(a) The longevity of flies without a blood feed and the influence of a blood feed on ovarian development were studied. In a series of about 100 flies separated immediately after fertilisation was actually observed, it was found that the maximum length of life without a blood meal and under the conditions of the experiment, was 12 days. During this period there was no development of the ovaries, there being very little difference observed by dissection between flies at the beginning and the end of the experiment.

Table II gives the results.

Table II

Number of flies used	Number of deaths occurring on different days											
	1	2	3	4	5	6	7	8	9	10	11	12
109	7	10	12	16	8	3	12	8	19	2	9	3

That blood was essential for the development of eggs was also ascertained in another series where flies after their first feed on human being were kept alive on fruits. The first batch of eggs was laid on the fourth or fifth day after the blood meal and no further oviposition took place although some of the flies were alive for another twelve days. When the flies were given an opportunity of a further blood feed, once or oftener during this period, one or two further batches of eggs were laid by them.

(b) Experiments conducted to see how long flies would survive on fruit juice after an initial blood meal showed that 13 days was the maximum period during which they could be kept alive in the laboratory. Table III shows the survivors in various batches for a period of ten days after the blood feed.

Table III

Number of flies put in	Number of flies alive after ten days
45	32
19	5
49	21
44	15
42	nil
32	22
40	20
40	15
41	14
30	15
67	7
94	10
83	20
52	17
87	17

(c) Catches in the early part of the night consisted mostly of unfed flies; males were also more plentiful at this time. Those caught in the early hours of the morning were invariably engorged ones.

(d) So far, all attempts to collect sandflies outside buildings have ended in failure. Sweepings of the undergrowth outside houses wherein sandflies have been collected have given miscellaneous and otherwise plentiful catches of other insects and spiders but not sandflies.

(e) Attempts were made to find out whether sandflies in nature feed on anything other than blood. All flies caught in their natural surroundings were examined by dissection and those without any trace of digested or undigested blood were examined under the microscope for any fluid that might be present. Flies with undeveloped ovaries were mostly newly hatched ones and only bubbles of gas were present in their guts. A small number of these had, however, well developed ovaries suggesting that they had fed earlier and had digested their blood meal when they were caught before they could take to their breeding grounds.

(f) In a specially selected place investigations regarding the prevalence of flies on successive days were made with a view to note what the effect of spraying

on the sandfly population would be. The number of flies collected daily during the first ten days were 9, 3, 10, 3, 4, 1, 6, 6, 4, 5 respectively. After an interval of 10 days collections for another week were carried out giving the following catches—8, 9, 7, 7, 4, 6, 5, 4. To ensure uniformity the catches were made by the same collector all through the experiment.

MISCELLANEOUS.

Up to date, from the beginning of the year 66,408 flies have been bred in the laboratory for infection experiments with human volunteers and experimental animals. Altogether 8,703 flies were caught in nature from dwellings and cattlesheds. Blood from one hundred and fifty suspected cases of kala-azar were examined, both by the aldehyde test and by microscopic examination. Of these 35 proved to be positive for malaria, and 73 for kala-azar by the aldehyde test, in 40 of which parasites were demonstrated in the peripheral blood by the routine method consisting of an examination of four slides per case. Twenty-three were negative by these tests and the rest were doubtful ones. No correlation was observed between the number of parasites present in the peripheral blood and the percentage of infection in flies fed on such blood. Two cases with big spleens and complicated with cancrum oris did not show any development in flies fed on them, although one of the cases showed a fair number of parasites in the peripheral blood.

38. Enquiry on the algae present in water reservoirs and their effect on purification methods and on public health under the Director, King Institute, Guindy, Madras.

The main lines of research were as follows :—

1. studies on the control and elimination of algal growths in water supplies,
2. determination of the optimum dose of suitable algicides for the more troublesome species of algae,
3. limnological studies on the large Stanley Reservoir at Mettur,
4. observations on the vertical stratification of plankton algae, dissolved gases, and on temperature in stored river water, and
5. studies on the influence of algae on the oxygen absorbed values (Tidy's test).

ALGICIDAL TREATMENT.

Systematic application of algicides to the water stored in large earthen reservoirs was continued during the year at Salem, Ellore, Cocanada, and Chidambaram, and to the water in the wells at Dindigul. It was found that under conditions of stagnation, decreasing water level, and high temperature with long hours of sunshine obtaining during summer months, the algal growths were comparatively excessive. Blue green algae, chiefly *Microcystis*, *Oscillatoria*, *Anabaena*, and *Anabaenopsis*, formed the bulk of the plankton occurring frequently in such large numbers as to import a distinct greenish colour to the water, and seriously affected the efficient working of the filters. Marked seasonal variations in the composition of the plankton algae in these reservoirs were also found to occur, a brief diatom and euglena-phase occurring during the period

following heavy rains, rapidly changing to a protococcale-phase during the dry cool season, which in turn was succeeded by a blue-green algae-phase in summer.

Systematic application of copper sulphate, or copper sulphate and chlorine whenever necessary, was found to be very effective in checking the profuse growth and development of the offending organisms, in the elimination of the foul odour produced in the underlayers of the filters, and in maintaining comparatively longer and more efficient filter-runs.

Experiments conducted in the laboratory, and in the field, showed that most of the blue-green algae were killed with 0.3 to 0.4 ppm. of copper sulphate in 8 to 10 hours. *Microcystis*, however, was less affected by copper sulphate alone than by a combination of this salt with chlorine. Of the protococcales which were previously observed to be very resistant to the action of copper, it was found that *Ankistrodesmus*, *Pediastrum*, and *Selenastrum* were rapidly killed with 0.2 ppm. each of copper sulphate and chlorine. Diatoms excepting a species of *Melosira* have never occurred in sufficiently large numbers in the tanks and reservoirs under investigation to cause any pronounced disturbance or resistance to filtration and other purification processes.

The sprinkling of small crystals of powdered copper sulphate along the shallow margins of tanks was tried at Chidambaram and Ellore and also in the large impounding storage reservoir at Salem, as an additional measure of algal control. The results have been satisfactory and are being carefully watched.

Limnological studies on the large Stanley reservoir at Mettur were continued. The findings of the previous year were confirmed. It was observed that the diatom-phase of the annual plankton cycle which starts soon after the advent of the heavy silt-laden flood water, lasts quite till the end of December, when the water becomes clear and almost transparent. The data obtained so far regarding thermal and gaseous stratification in the lake have not been conclusive.

Confirmatory studies were carried out on the diurnal changes in the temperature and gas content of river water stored in masonry tanks. It was established that under conditions of storage a marked thermal stratification did occur during the day and was probably destroyed by night, thus resulting in a complete daily overturn of the stored water. This is an important finding from the point of view of practical water supply maintenance in the tropics. It was also observed that the migration of the algae downwards during the period of very bright sunshine resulted in a maximum oxygen saturation at depths of 1 to 1½ feet below the surface, manifestly the result of photo-synthetic activity of the algae.

Further studies on the relation between the organic matter content (Tidy's test) and the algae plankton with particular reference to the amount of organic matter contributed by the algae, were carried out using suspensions of single algal forms, like *Oscillatoria*, *Anabaena*, *Staurastrum*, *Cosmarium*, *Englena*, *Phacus* and *Microsystis*. The values obtained appeared to be directly proportional to the number of organisms present in the case of the first four organisms, while in the case of the others, a conclusive finding could not be obtained.

A detailed study of the organic growths occurring inside an infiltration gallery at Negapatam was started in October 1942. The growths were dark-brownish in colour, and were found loosely adhering to the sides of the collecting well of the gallery. They were composed of loose masses of filamentous forms enclosed in a gelatinous matrix ; they belong to the group of Iron Bacteria.

The presence of these growths inside a closed water supply system of a gallery was recorded for the first time. Studies on the nature and life history of these organisms and on the remedial measures to be adopted have also been undertaken. These growths, if allowed to remain unchecked, can produce serious results by choking the supply system.

39. Enquiry into the pathology and pathogenesis of splenomegaly under Dr. D. Govinda Reddy at the Medical College, Madras.

A study of spleen, liver, bone-marrow and peripheral blood changes in rabbits subjected to manganese chloride intoxication was made. This inorganic poison has been extensively used to induce cirrhosis of liver. Changes in the spleen were recently studied and a pre-cirrhotic toxic splenomegaly ultimately ending in periarterial fibrosis and fibrosis of the pulp was noticed and resemblance to 'fibroadenie' of Banti's spleen suggested. These changes in the spleen in the absence of progressive anaemia and leucopenia are not helpful in the understanding of the pathology of splenic anaemia. So far none of the workers has made any reference to blood and bone-marrow changes. The present investigation was mainly directed to the study of these changes.

Method and material—Thirtyfive young rabbits were used for the investigation, of which 9 were control animals. These were divided into two batches. The first group was injected with large doses of 1% manganese chloride solution in saline. The second group received smaller doses of the chemical of 0.5% strength. The injections were given intramuscularly at specified intervals after a preliminary comprehensive haematological examination. This comprised R. B. C. count, haemoglobin estimation, total and differential leucocyte counts, platelet count and reticulocyte percentage. The blood counts were repeated twice weekly at first and later once every fortnight. The animals were killed at intervals and the changes in spleen, liver and bone-marrow were studied.

Splenic changes—There was a moderate increase in the weight of the spleen in most of the cases, but it was not uniform. There was no noteworthy macroscopic change. Spleens of animals that received large toxic doses showed degenerative changes in the malpighian follicles and pulp syncitium (Billroth cords). The malpighian follicles showed uniform atrophy with irregularity of peripheral zone. No germ centres were to be seen. The lymphocytes were more compact and deep staining. Nuclear changes like karyorrhexis and pyknosis were seen in some of the cells, chiefly in the marginal zone of the malpighian follicles. Here early fibroblastic reaction was noticed. The wall of the central artery of the malpighian body showed slight thickening with swollen endothelial lining. The pulp cords were thickened and the pulp cells presented a swollen appearance due to granular degeneration and coagulative necrosis of the cytoplasm. A few nuclei of the pulp cells showed degenerative changes similar to those seen in the periphery of the malpighian follicles. These changes were diffuse and not delimited to the vicinity of the malpighian bodies. Due to swollen Billroth cords, the sinusoids were partly obliterated. The reticulum cells lining the spaces were intact with a few of them protruding prominently into the lumen due to a swollen condition. Large macrophage cells containing haemosiderin pigment and occasionally entire erythrocytes were seen both in the pulp mesh and sinusoidal spaces. Haemorrhages were rare. Siderotic nodules were never met with.

Spleens of animals that received repeated small doses and which died early, showed changes similar to those described above ; but those that survived long showed more of proliferative and regenerative changes. The lymph follicles were prominent, the pulp cords almost normal and sinusoids distended. Haemosiderin laden phagocytic cells were found in some of them. The central artery as well as the branches appeared normal. The capsule was slightly thickened and the trabeculae were prominent. The reticular mesh of some of the malpighian follicles was increased as shown by special methods of staining.

Liver changes—Livers of animals that received large toxic doses showed fatty degeneration and patchy necrosis chiefly in the periphery of the lobules. This resulted in an early monolobular cirrhosis due to atrophy of liver cells and connective tissue formation. Animals that survived long with small repeated doses showed no gross changes but animals of this group that died early showed hepatic damage.

Blood changes—Haematological examination in all the animals failed to show appreciable changes. The erythrocyte counts and haemoglobin content showed fluctuations within normal limits, and none of the animals showed even a moderate degree of anaemia. Immature forms of erythrocytes were never met with in the peripheral blood, nor was there reticulocyte increase to indicate rapid regeneration of erythrocytes. In the majority of the animals there was a rise in the leucocyte counts. Inflammatory reaction of the tissues at the seat of injection may be responsible for this.

Bone-marrow—Both macroscopic and microscopic studies of bone-marrow in selected bones as femur, tibia, sternum and ribs were made. Stained smears were utilised for differential counts, as identification of individual cells in sections was not always easy. The sections of bone-marrow provided material from which the total amount of haemopoietic tissue was estimated. The bone-marrow of some of the animals injected with toxic doses of the chemical showed congestion and degenerative changes of a few fixed mesenchymal cells which form the reticular stroma. The nuclei of megakaryocytes showed condensation. Scattered all over were found large phagocytic cells containing haemosiderin pigment. In the case of animals that received small doses, the bone-marrow appeared more cellular, suggesting a hyperplastic reaction. There was no increase of argyrophil or collagenous fibres. There was no evidence of either degenerative changes of bone-marrow or hyperplasia of erythropoietic or granulopoietic tissue. The quantity and distribution of fatty marrow appeared unaltered.

40. Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

Isolation of anaerobic organisms.

During the last eleven months, the work of isolation of anaerobic organisms, from various sources, has been continued. Material from 93 new cases was made available making up a total of 158 cases studied so far. The distribution of these new cases was as follows :—

	Number of cases
Casualty wounds	21
Cases of gas-gangrene	11
Cases of tetanus	6
Surgical cases	15
Post-mortem material	12
Samples of manure	6
Samples of horse-dung	2
Samples of guineapig stools	5
Samples of human stools	8
Sample of catgut	1
Material from other sources	6
Total number	93

STRAINS OF ANAEROBIC ORGANISMS.

Thirtytwo new strains of anaerobic organisms were isolated from these cases. Some of these were identified after studying their morphological, cultural and biochemical characters. Animal pathogenicity of these organisms was tested by using ' white mouse ' as a test animal. The incidence of these new strains was as follows :—

Clostridium welchii	5
,, tetani	4
,, histolyticum	2
,, sporogenes	5
,, septique	1
In the process of identification	15
Total number ..	32

Cases of tetanus—Material from 6 definite cases of tenanus was obtained. It became possible to isolate *clostridium tetani* in three cases. From two cases it could not be grown. Material from the sixth case is in the process of study. A strain of *clostridium tetani* along with *clostridium sporogenes* was isolated from a case, of bullet wound, in which a suspicion of gas-gangrene group of infection was entertained. Similarly 4 strains of anaerobes resembling *clostridium tetani* have been isolated from material obtained from surgical cases. They are in the process of identification.

Eight different samples of horse-dung and manure were tested for the presence of *clostridium tetani* in them. From none of these could it be grown.

Cases of gas-gangrene—During this year material obtained from 11 definite cases of gas-gangrene was submitted for bacteriological investigation to isolate the organisms responsible for this infection. It was noted that :—

Clostridium welchii alone was responsible in ..	.. 3 cases.
,, welchii along with clostridium sporogenes in	.. 1 case.
,, septique with clostridium sporogenes in	.. 1 case.
,, histolyticum along with clostridium sporogenes in	1 case.

Material obtained from 5 cases of gas-gangrene is in the process of study. In addition to this, 13 different samples of human and guineapig stools, were studied to isolate clostridium welchii from them. This work is undertaken to

study the antigenic differences between clostridium welchii isolated from various sources as also, to study its incidence of toxigenic and non-toxigenic strains from such sources. A strain of clostridium welchii was isolated from a sample of human stools.

TOXIGENIC CAPACITY OF CLOSTRIDIUM WELCHII.

In order to study the toxigenic capacity of the strains of clostridium welchii a standard toxigenic strain of Cl. welchii S. R. 12 (kindly supplied by Colonel Sokhey of the Haffkine Institute, Bombay) was used as a control. Five strains were tested to study their toxigenic capacity (after ascertaining that they were pathogenic). It was found that only one strain (out of the five) yielded toxin. The toxin extracted on repeated occasions, from this strain was less powerful than that of the strain of Cl. welchii S. R. 12.

IMMUNISATION OF RABBITS.

Rabbits are being immunised against toxins of strains of Cl. welchii S. R. 12 and 53, with a view to producing purely antitoxic sera from different strains of Cl. welchii. One of the rabbits immunised against the toxin of Cl. welchii S. R. 12 was bled through the carotid artery. Clear serum obtained is being tested for its antitoxic strength. So far 0.01 of the serum given intraperitoneally protected mice against 0.2 cc. of the toxin of strain of Cl. welchii S. R. 12 injected intravenously (0.2 cc. dose of toxin found to be the M.L.D.). It is being tried in further dilutions.

It is interesting to note that no pathogenic anaerobe was isolated from deeper portions of fresh casualty wounds from the road side. Almost all the strains of pathogenic anaerobes have been, either, isolated from definite cases of such infection, or from old infected wounds. A few strains of clostridium tetani have been isolated from surgical cases in which there was no clinical suspicion of such an infection. This emphasizes the value and importance of the routine use of prophylatic doses of anti-tetanic-serum.

Since the beginning of the enquiry 19 definite cases of gas-gangrene were studied and it was found, that Cl. welchii was mainly responsible for the infection. All the recently isolated strains of Cl. welchii, which were proved to be pathogenic, were tested for their toxigenic capacity and so far, only one strain has proved to be toxigenic. In the light of this fact, it may be stated that the other strains of Cl. welchii were pathogenic by virtue of their invasive capacity. Anti-bacterial rather than antitoxic serum is likely to be of use in treatment of cases infected by such atoxic strains (though pathogenic) of Cl. welchii. Experiments are being planned to study the value of chemo-therapy, anti-bacterial and anti-toxic serum alone or in combination in treatment of Cl. welchii infection in experimentally infected animals.

41. Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta.

METHOD.

The closed systems used for pooling and filtration are adaptation of those described by Hayes, Dhall and Sami (Ind. Med. Gazette, 1942, Vol. 77, p. 197).

Laboratory.

The processing laboratory in Calcutta is air conditioned and the air injected into the room is 100% fresh air filtered through a " replacement type filter " and again through a " visco filter ". The rooms are sprayed every morning and at the end of the day with 2% " Dettol " by means of a spray gun at a pressure of 10–12 lbs. Besides these precautions the chamber in which the filtered serum is finally bottled is steamed for an hour in the evening previous to filtration.

When large batches of serum have to be processed the sterility of the room is to be considered an absolute necessity.

Pooling.

Samples of clotted blood are kept at 4°C–6°C for 72 hours, after which the serum is aspirated out in a closed system and pooled irrespective of group and Kahn tests. Bottles showing unusual haemolysis or fat are discarded.

Pooling is done in Winchester bottles which can hold serum from 18 – 20 donations of blood. The number of Winchesters used in a single pooling depends upon the amount of serum awaiting processing. However 18 – 20 samples are required for one pooling because the agglutinin titres of filtered sera, as found in the 95 batches filtered so far, have been in practically all cases $\frac{1}{2} - \frac{1}{4}$. A titre of 1/16 occurs in less than three per cent of cases. Arrangements are being made to pool in 10 litre bottles, when it will be possible to pool about 80 samples of sera.

Two assistants, aseptically dressed, do the pooling. A third assistant supplies the bottles of blood after flaming the top and removes the used ones, and a fourth takes away the used needles and cleans them. Pooling needles are autoclaved in paper packets containing 12 in each, and one such needle is used for each bottle. The air inlet is the same throughout, and it is flamed each time before putting into the bottle. Each of the two workers has his own air-filter, consisting of 12″ length of tube packed with cotton and sterilized.

About 15 inches of vacuum is used for pooling. The average speed of each worker is 30 – 40 bottles per hour. It takes half an hour for fitting up the apparatus, another half an hour for dismantling and an hour for washing and cleaning.

After pooling the bottles of serum are aseptically stoppered and put in cold storage for about 18 hours.

Clarification.

Pooled serum is clarified by passing through a pad made from paper pulp. The diameter and thickness of the pads used are respectively 5″ and about 2″. Two such pads are prepared inside two clarifying chambers and put in trunks along with other materials for sterilization. After sterilization and before passing serum through the pads, 3 litres of saline are sucked through them. After this, it has been found possible to clarify 16 – 20 litres of serum through each pad.

Filtration.

Filtration is carried on with positive pressure and the whole apparatus is an entirely closed system throughout processing.

Fourteen cm single pad seitz filters are used and it is possible to filter 4 litres through each pad. Lately 8 – 10 filters have been used for filtering 30 – 40 litres at a time. The pressure is gradually raised to 5 – 6 lbs and the average speed is about 2 litres per hour per pad when the pressure is at the maximum limit, but there is a tendency for the speed to slacken during the later stages of filtration.

To make the process of filtration and bottling of the filtered serum continuous, two-three-litre bottles are connected in parallel to receive the filtered serum, so that when one is being emptied into the transfusion bottles, the other is being filled with serum coming out of the filters. Each bottle has its own cotton filter to filter the sterile air used for forcing out the filtered serum.

Filling and capping.

Transfusion bottles are filled under a hood, which is flamed from time to time. After filling, the aluminium cap is put on the bottle under a flame. The metallic cap is then swabbed with a solution of 2% carbolic acid and 10% glycerine and a viscap is slipped on it. The viscaps are preserved in 5% formalin solution and before use they are taken out, washed in sterile distilled water, and soaked in 1% carbolic acid solution about $\frac{1}{2}$ – 1 hour before use.

Sterility tests.

For every 3 litres of filtered serum samples are taken out for sterility tests. 5 – 10 cc. are run into 50 c.c. glucose broth contained in a transfusion bottle which is then treated like any other serum bottle. It is afterwards incubated at 37°C for 6 days and then kept at 25° – 27°C for two days and examined. Other samples of serum are taken for additional sterility tests which consist in adding 5 c.c. to flasks containing 50 c.c. glucose broth and incubating aerobically as well as anaerobically. Tested this way the percentage contaminations during September to November was 8 or 4.9 per cent, the total number of samples tested being 162. On retesting all the 8 samples were found sterile, so that there was really no contamination during these 3 months.

Storage.

After filtration all bottles are stored in the laboratory at about 25° – 27°C for 2 – 3 weeks. The bottles are then individually examined against a light and if found quite transparent, are transferred to cold room at 4°C – 6°C for storage.

Output.

At present with 5 assistants 100 – 120 litres of serum per week are being processed.

42. Enquiry into the occurrence of ascites in Hyderabad under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan.

A study of the post-portem examination records of 340 cases in the Pathology Department of the Osmania Medical College, showed that ascites was present in 86 cases (25.29%). The ratios of ascites cases to the total number of post-mortem examinations in K. E. M. Hospitals, Vizagapatam, and the

Massachusetts General Hospital were 27.21% (277 cases out of 1018 post-mortem examinations) and 10.1% (224 cases out of 2217 post-mortem examinations) respectively. The principal disease conditions associated with which this was found as compared with the figures from these two institutions are shown in the table below :—

Table I

S. No.	Disease.	Percentage of total cases.		
		Massachusetts.	Vizagapatam.	Hyderabad Deccan.
1.	Cardiac weakness	.. 43.75	19.1	23.25
2.	Diseases of peritoneum	.. 27.04	22.79	12.78
3.	Renal disease	.. 11.5	5.14	11.62
4.	Cirrhosis of the liver	.. 10.26	25.72	29.2
5.	Pulmonary and intestinal tuberculosis	..	9.59	1.16
6.	Anaemias	.. ..	5.51	7.14
7.	Dysentery	.. ..	7.53	5.22
8.	Beri-beri	.. ..	1.84	1.16
9.	Cancer head of pancreas	.. ..	0.73	3.44
10.	Indefinite causation	.. ..	3.3	3.44

On examining the figures of in-patient admissions in the Osmania Hospital for the two years 1940 and 1941 it was found that ascites was more common than previously suspected, practically one in every sixtysix patients seeking in-door treatment showing this condition as is shown below :—

Total number of in-patients for the period=32,364 (17,869 males and 14,495 females).

Total number of ascites cases for the same period =490 (263 males and 227 females).

A comparison of the clinical diagnoses of these 490 cases with that of the 3,074 Massachusetts cases is shown in the following table.

Table II

S. No.	Diseases.	Massachusetts (3074 cases) Percent	Hyderabad Dn. (490 cases) Percent
1.	Cardiac insufficiency ..	45.45	27.55
2.	Renal diseases ..	21.69	10.6
3.	Hepatic Cirrhosis ..	10.05	28.5
4.	Peritoneal tuberculosis ..	8.59	5.5
5.	Anaemias ..	0.49	6.73
6.	Unknown causation ..	..	18.36

The tables above show that clinically diseases of the liver are much more important in Vizagapatam and Hyderabad than cardiac disease which is the most important in Massachusetts. There is also a fairly large number of cases

at Hyderabad in which no definite cause is attributable. As the proportion of these type of cases is clinically much larger than in post-mortem examinations, it is probable that a fair number of them recover. This is also borne out by clinical observations. Detailed analysis of the clinical records of this group of cases showed that out of a total of 90 cases there were 58 males and 32 females ; 58 Hindus and 32 Muslims. The majority of the sufferers belonged to the rural working-classes in the active period of life or over middle-age. Religion does not seem to make much difference.

In response to a circular, figures of cases of ascites were received from 50 hospitals and dispensaries in the state. Although the total number is comparatively small two important facts are brought out. Hyderabad State can be roughly divided into two parts, the western Marathwada where the staple diet is jawar, and the eastern Telangana where the staple diet is rice. The returns received, do not show that ascites is confined to or is more prevalent in the rice eating Telangana districts than in the jawar eating Marathwada. Cases of ascites (not attributable to organic diseases of the heart, kidney or liver etc.,) which have recovered and remained well have been reported by several Medical Officers. Cases of ascites (1) with cirrhosis of the liver (clinically diagnosed) and (2) without cirrhosis or gross cardiac or renal disease etc., with or without anasarca, as well as cases of oedema without ascites have been studied at the Osmania Hospital. Several cases of ascites and oedema un-associated with easily recognisable organic disease were seen. Their clinical picture and progress under hospitalization and treatment are described below.

The patient is weak, hardly able to walk and in cases of ascites suffers discomfort on sitting or lying down. The pulse rate does not differ markedly from normal, the blood pressure is low, 105/75 mm. Hg. or even lower figures being not uncommon. The heart may be dilated in some cases with distress of breathing on slight exertion, while in others it appears normal. The lungs on physical examination are usually clear. The appetite is fairly good. The bowels are constipated but there is occasional un-accountable diarrhoea. There may or may not be oliguria ; usually there is no albumin in the urine or only a trace and no casts or blood cells are found. The stools are positive for hookworm ova in a few cases. There is no appreciable tenderness of calf muscles and no anaesthesia. The blood picture shows a varying amount of anaemia of a hypochromic type without abnormal types of cells.

The blood urea and cholesteral were found within normal limits.

The kidney function was fairly good as indicated by the phenolsulphanephthalein test.

Treatment—The patients were placed on a salt-free baker's wheat bread and milk (Bread 20 oz, Milk 21 oz, with butter 1/8 oz daily, with sugar and tea ; caloric value about 2200) with a sweet lime ; yeast powder about half an ounce made up into a confection with syrup was given with the bread. In the initial stages an occasional injection of a mercurial diuratic (Neptal) was given. The routine mixture was the following :

Pot. acetas.	..	.. gr. X
Spt. aethris nit.	..	.. mm XX
Tinct. digitalis.	..	.. mm X to XV.
Inf. scoparii.	..	.. ad. oz. 1

oz. 1 td.

A dose of magnesium sulphate was given when necessary.

Course—The response to this line of treatment was very satisfactory. The oliguria when present was replaced by free diuresis. The ascites and oedema rapidly diminished without paracentesis or the use of Southey's tubes ; there being a loss of body weight of $\frac{3}{4}$ to $1\frac{1}{2}$ lbs. per day. In less than a week the patient was able to sit up without discomfort and in a few days more able to walk about. Later, when a more varied diet including salt was given the oedema did not recur, the improvement was maintained and real weight without oedema gained. The course of most of these cases is afebrile. After discharge, however, a relapse of the condition (oedema) was noticed.

Conclusions :—

Subject to correction in the light of further observations the data so far obtained indicate :—

1. That ascites with or without oedema (or oedema without ascites) is a much more common condition in Hyderabad State than was suspected.

2. Its occurrence is not confined to those whose diet is predominantly rice or who are addicted to alcohol. It occurs mostly in the rural labouring classes, in persons over 25 years of age with no special reference to religion.

3. The picture does not correspond to the text-book descriptions of beri-beri.

4. On the whole the condition seems to results from general malnutrition rather than a particular kind of diet.

CLASSIFICATION OF THE CASES.

1.	Ascites with oedema		.. 14 cases.
	1. Associated with cirrhosis of the liver ..	6	
	2. Nutritional deficiency..	4	
	3. Cardiac decompensation	2	
	4. Ch. nephritis	1	
	5. Pulmonary tuberculosis	1	
		14	
2.	Ascites without oedema		.. 7 cases.
	1. Associated with cirrhosis of the liver	2	
	2. Nutritional deficiency	1	
	3. Nutritional deficiency (suspected tubercular peritonitis)	2	
	4. Secondary syphilis	1	
	5. Myocardial degeneration (? Beri-beri) ..	1	
		7	
3.	Oedema without ascites		.. 6 cases.
	1. Nutritional deficiency..	4	
	2. Beri-beri ?	1	
	3. Malaria with nephritis	1	
		6	

Thus of the 27 cases of ascites with or without oedema or only oedema, the cases attributable to various causes are :—

1.	Cirrhosis of the liver	8 cases
2.	Beri-beri ?	2 „
3.	Nutritional deficiency	9 „
4.	Nutritional deficiency with (suspected tubercular peritonitis)	2 „
5.	Cardiac decompensation	2 „
6.	Chronic nephritis	1 „
7.	Malaria with nephritis	1 „
8.	Secondary syphilis	1 „
9.	Pulmonary tuberculosis	1 „
		27

The diet of the 11 cases of ascites or oedema due to nutritional deficiency (including 2 of Beri-beri) was as follows :—

Chiefly rice	6 cases.
Jawari and rice	5 „

43. Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

The effect of neurotoxin, haemolysin and choline-esterase isolated from cobra venom on heart, blood pressure and respiration.

The m.l.d. of cobra venom used in our experiments is 0.1 mg. and that of the neurotoxin which has been isolated and will be referred to as *neurotoxin* (*R*) is 0.01 mg. The haemolysin and cholin-esterase are each weight for weight about 11 times more active than the crude cobra venom. It has been observed by us that injection of 2 mgs. of crude cobra venom per kilo. of the body weight of rabbits causes, after a preliminary rise, a sharp fall of blood pressure and stoppage of heart. The effect of *neurotoxin* (*R*) on the blood pressure of rabbits has been found to be practically negligible, the dose ranging from 0.1. mg. to 8.5 mg. per kilo. The most striking effect of *neurotoxin* (*R*) is its action or respiration. On injection of 0.2 mg. of this neurotoxin per kilo. into a rabbit there is a slight rise of blood pressure and the respiration gradually diminishes and in 9 minutes it stops altogether. With the failure of respiration the blood pressure rises due to asphyxia. On the administration of artificial respiration the blood pressure comes back to the original normal level and remains steady at this level so long as artificial respiration is maintained. The second dose of *neurotoxin* (*R*) into a rabbit under artificial respiration when breathing had stopped as a result of previous dose of neurotoxin, shows the usual slight effect on the blood pressure which remains steady. On the other hand injection in the same way of crude cobra venom (2 mg. per kilo) after neurotoxin into rabbits under artificial respiration causes a sharp rise of blood pressure followed by a rapid fall and stoppage of heart. It is thus evident that crude cobra venom

contains at least two neurotoxins, one paralysing the movement of the heart and the other that of the respiratory system.

In the perfused toad's heart haemolysin produces augmentation and depression and irremovable irregularity and ventricular block without causing cardiac failure. Haemolysin in weak doses, does not affect the circulation or the respiration, but in heavy doses causes failure of both. Choline-esterase in high concentration slightly stimulates the perfused toad's heart but has practically no action on blood pressure or on respiration of rabbits.

Effect of snake venom on the oxidation of lactic acid, succinic acid etc. in tissue cells.

It has been reported previously that snake venoms specially those of cobra (Naja Tripudiana) and Krait (B cerelus) strongly inhibit the conversion of glucose into lactic acid in the brain cells of pigeons. In continuation of this work the action of cobra venom and some of its constituents on the oxidation of lactic acid in the brain cells of pigeons has been investigated. It has been found that crude cobra venom when added to a suspension of brain cells in Ringer-Locke solution mixed with sodium lactate, a marked inhibition of the oxidation of the lactic acid is observed. It has also been observed that haemolysin isolated from cobra venom does not inhibit the oxidation process to any appreciable extent.

Effect of snake venom on glycolysis in the carcinoma cells produced in rats.

Several batches, each consisting of 15 to 20 rats are being injected regularly with carcinogenic hydrocarbons like methy-cholanthrene. So far tumours have developed in three rats only. Preliminary experiments which have been carried out with these tumour tissues show that cobra venom has got marked inhibitory action on the conversion of glucose to lactic acid in such cells under anaerobic conditions.

Action of snake venom and their constituents on the synthesis of choline in the brain cells of rats.

As acetyl-choline mediates in the transmission of nerve impulse, a study of the effect of cobra venom and its constituents, on its synthesis in tissue cells will be of great interest. Investigation on this problem using brains of rats has been started. The acetyl-choline is being estimated by the usual method of measuring the contraction of the dorsal muscle of leeches. It has been found that the synthesis of acetyl-choline in the brain cells of rats is considerably inhibited by crude cobra venom.

44. Enquiry into the isolation of atropa from *dhatura* under Dr. J. N. Ray at the Delhi University Laboratories, Delhi.

Examination of the black and white varieties of Datura Fastuosa and Datura Alba has been completed. As is well known these grow abundantly in many parts of India and it was, therefore, necessary to carefullly investigate if any atropine could be isolated from these plants. Plants collected from Bengal, Delhi, Hardwar and the Punjab have so far been examined. The

total alkaloid content varied from 0.2 to 0.4% on the basis of dry leaves. Qualitative tests indicated the presence of both *hyoscyamine* and *hyoscine*. It has moreover been found possible to isolate hyoscine hydrobromide both from Dhatura Fastuosa V niger and Dhatura Alba, but in very poor yields. The yield of atropine from a specimen of Dhatura leaves growing in the Shivalic Range amounted to nearly 0.05%. For large scale processes such specimens would be consequently uneconomical.

From the investigations carried out by us it appears that the commoner varieties of Dhatura can hardly be considered as suitable sources of atropine. But the prospects of preparing hyoscine do not appear to be bad.

45. Construction of Pilot Plant for drying human plasma or serum at the All-India Institute of Hygiene and Public Health, Calcutta.

A plan of a plasma or serum drying plant designed by the sections of Physiological Hygiene and Public Health Engineering, All-India Institute of Hygiene and Public Health was submitted at a meeting of a special committee on blood banks held on 13th December 1941 in the office of the Director General, Indian Medical Service, in the Secretariat building, New Delhi. A discussion followed, chiefly on the question of utilising silica gel as a dehydrating agent which was an important feature of the design, as some workers expressed doubts about silica gel being able to produce a dried product with a residual moisture of 1% or less. The members desired proof of this vital point from laboratory samples and the All-India Institute of Hygiene and Public Health undertook to establish this point beyond doubt.

Accordingly, a sample was produced on 12th January, 1942 which had a moisture content of less than .4%. An independent estimate reported on 4th February, 1942 gave a moisture content of about .6% in the sample. As these results were better than the specification of 1% required, authority was given on 17th February 1942 to proceed with the construction of the plant and an allocation of Rs. 13,000/- was made by the Indian Research Fund Association for the purpose.

The plans were revised in the light of local conditions and materials available ; and fresh drawings were prepared. Through the kindness of Mr. J. M. Robertson, I. P. O. S. S. of the Department of Supply, we were introduced to Mr. Longley, Chief of the firm of Messrs. Gresham and Craven, specialists in the manufacture of vacuum brakes and equipment. Mr. Longley took enthusiastic interest in the design and put the resources and talents of his workshop and staff at our disposal for the construction of the plant. Some parts of the plant were built at the E. I. Ry. Workshop, Lillooah.

The prefreezing equipment was originally designed for using dry ice as the refrigerant, in accordance with the practice employed in other types of plant. However, on reconsideration, this design had to be changed, as the evaporation of large quantities of dry ice in an air-conditioned room would have been attended by the risk of suffocation of workers on account of excessive proportions of carbon-dioxide in the air. Alternative methods of refrigeration were thought of, and it was finally decided to make the plant self sufficient by using a mechanical refrigerator and calcium chloride brine. Mr. Driver of the firm of Magnolia Dairy Products was consulted, and he very kindly assisted us by building the prefreezing tank and installing an ammonia compressor for our requirements.

Meanwhile, the question of obtaining sufficient silica gel for our pilot plant was becoming a problem. Thanks to the Rockefeller Foundation, Mr. Dyer cabled with commendable forethought for 50 lbs. of silica gel to America. However a further 100 lbs. was required to be on the safe side. Several successful samples were prepared in the department of Physiological Hygiene, All-India Institute of Hygiene and Public Health, which proved superior to all imported specimens. At this juncture, Professor J. N. Mukherjee of the University College of Science, Calcutta, very kindly offered to manufacture 50 lbs. if he was assisted with a small grant of money. The samples produced in his laboratory were also quite satisfactory and funds were made available to him according to his requirements from June 1942. We were able to get 50 lbs. from Imperial Chemicals and another 50 lbs. through the kind offices of Mr. Hardman of the English Electric Co. from the Calcutta Electric Supply Corporation.

The electrical portions of the plant, such as electrical ovens, thermocouples etc. were designed and manufactured for us by Mr. Basu of the Indian Transformer Co.

By about the end of July Mr. Longley was ready for tests on the plant that had been assembled in his workshop. The most essential test was the ability of the plant to retain a high vacuum. It was gratifying to note that even on the first test on 28th July 1942 the Pulsometer pump produced a vacuum of about 400 microns in 20 minutes and the leakage amounted only to a loss of vacuum of 150 microns per hour. This was encouraging and sufficient for our purpose. The plant was dismantled and reassembled in the institute, in close cooperation with Messrs. Driver and Longley and their staffs and the central P. W. D. The installation was completed and the first vacuum test was even better than at the Works. A vacuum of 150 microns was attained on 21st September. The prefreezer was also satisfactory. The temperature of -30°C was attained in the brine tank and all was set for the first run of desiccation.

The first charge of 6 litres of discarded serum was placed in the desiccator and after a period of 100 hours the samples were taken out. They showed a moisture content of nearly 2.80% and there were signs of thawing of plasma which probably took place when water was circulated at a temperature of 75°C in the early stage of desiccation. During this trial run, many practical details on the management of the plant were learnt by experience.

In the second run of desiccation completed on the 6th October 4 litres of fresh serum were used, and after 72 hours of desiccation a product was obtained with a moisture content of less than .9%. The dried serum was readily soluble in water. Thus the plant has come up to specification and is ready for production.

46. Leptospirosis enquiry under Dr. M. N. Lahiri at the Haffkine Institute, Bombay.

The work carried out included the following :—

Human cases of leptospirosis.

During the year under review fortyfive cases of fever with acute jaundice were studied. Apart from these, a fair number of cases also came under observation but are omitted from the list in view of the fact that the symptoms were not of an acute nature. Out of those fortyfive cases nine proved to be authentic

cases of leptospirosis, in each of which the diagnosis was established by laboratory methods. These positive cases do not, however, represent the true incidence of the disease in this city where only a few of the many cases that actually occur are admitted into hospitals. Further, recent observations suggest that many cases of the milder forms of the disease must have passed unrecognised while a few were wrongly diagnosed as other types of jaundice. The causal organism was isolated from six of the positive cases whereas in the remaining three the diagnosis was based on agglutination reaction and other serological tests.

The isolation can be best accomplished during the early part of the disease by blood culture but this method was found to have limited application since the majority of these cases came under observation, when Leptospirae had already disappeared from the blood. In one instance only was it possible to cultivate the blood on the seventh day of the illness. It yielded a good growth of leptospira. In five of the cases the isolation was made from urine by means of animal inoculation. Contrary to the findings of many workers, the method of systematic daily inoculation of urine into young guineapigs proved to be of definite value in the present study. This method no doubt is a laborious one and involves the sacrifice of a large number of animals. On an average eight samples of urine from each case were examined, and positive results were obtained only six times. It was evident that many samples were not infective and in two instances samples showing Leptospirae under the dark-ground illumination failed even to produce infection in animals. The strains of leptospira isolated all belonged to the " classical " group and so far this was found to be the only serological type responsible for the infection.

Among the serological tests, agglutination reaction was found to be a method of great practical value. In every instance the diagnosis was pronounced long before the organism was isolated. Three samples of serum were tested from each case to determine the rise as well as the final titre. In one case interesting results were obtained. The sample of serum collected on the 8th day of the illness was put up against the Calcutta " Classical " as well as the local strains. The serum reacted to a titre of 1:100 against the local strains but showed no evidence of any agglutination against the Calcutta one. Subsequent tests showed marked rise against all the strains. This brought out an important fact that in performing the test strains isolated in the locality should always be incorporated and also that the tests should be repeated to note the rise of titre.

Non-icteric form of leptospirosis.

The occurrence of non-icteric type of cases has not as yet been recorded in this Country. At the suggestion of Major General Sir John Taylor particular attention was directed to this question. So far only one positive case has been recognised in which there was no evidence of external jaundice. The examination of the urine in this case also showed absence of bile. This finding shows that such type of cases must have been missed before, and if the interest of the medical profession could be stimulated, many more cases might be detected with laboratory aid.

Maintenance of the virulence of the cultures of leptospira.

It was observed that repeated subcultures resulted in a considerable loss of virulence. The virulence has, therefore, been maintained by passage through young guineapigs approximately every four weeks. In order to economize in

animals the Cryochem method (drying from the frozen state) was studied. The method proved to be very useful in case of bacteria. The substrata used were the defibrinated blood of guineapig and rabbit. After drying, the cultures were regenerated at varying intervals and were examined under dark-ground illumination. It was found that even within twentyfour hours after drying the leptospirae had lost their motility and showed no evidence of any growth when inoculated into fresh medium. Similar results were obtained with the guineapig liver emulsions showing leptospirae in considerable numbers. As no success was achieved the method was not considered suitable.

The suitability of mice for experimental infection.

This investigation was undertaken early in the year, to determine the susceptibility of mice (Mus Musculus) towards *leptospira icterohaemorrhagiae* in view of the recent observations of Larson in U. S. A. There is no doubt, obvious advantage in the use of this animal from the point of view of cheapness. The mice bred at the Haffkine Institute were employed. Two virulent cultures isolated from cases were selected. For each dose of the cultures, three white mice were used along with a young guineapig as a control. The mice were inoculated intraperitoneally with varying doses ranging from 0.2 to 0.6 cc. of the cultures whereas the control guineapig in each test received 1 c.cm. Out of the thirtysix mice used in these experiments only one showed evidence of infection, and the organism was recovered by culture of the hearts blood of the animal. The control guineapigs all died of the infection exhibiting typical post-mortem signs. Their liver emulsions also showed leptospirae in large numbers. These observations clearly indicated that mice are fairly resistant to the strains of leptospira isolated here.

Miscellaneous investigations.

1. Twentyone specimens of Sera obtained from the positive cases at various stages of the disease were stored in the refrigerator. They were retested after 350 days to demonstrate any drop in titre. For this purpose the same strains were used throughout. With the exception of two samples all gave strong agglutination and their titre almost approached the original titre.

2. It has been observed that in certain countries the agglutinins disappear rapidly after recovery. A retrospective diagnosis can be made of the past infection if the agglutinins persist for long. In order to obtain information on this point samples of blood were collected at an interval of two months from two positive cases of last year which could be traced. It was noticed that even after 240 days there was no significant drop in the titre. The titre obtained were 1 : 10,000 and 1 : 5,000 respectively against the classical strains. It was concluded from these limited observations that in all probability the immune substances do not disappear quickly from persons who have had the disease. To demonstrate the presence of agglutinins, tests were later made on seventyfive sera collected at random from the blood samples submitted to this Institute for Wassermann test. So far agglutinins could not be demonstrated in any of the samples except one which gave a doubtful reaction in a dilution of 1 : 10. Further tests are being carried out. This study has a bearing on the epidemiology of the disease.

REPORTS OF ADVISORY COMMITTEES

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 10 a.m. on 7th December, 1942, and considered the reports on malaria enquiries carried out during the current year and the proposals for 1943-44.

EXISTING ENQUIRIES.

Malaria Institute of India (research section).

The research activities of the Institute have necessarily been greatly curtailed owing to abnormal conditions brought about by the war. Six intensive courses in malariology, each lasting four weeks, have been held during the year. Among the 152 students who attended these courses were 4 officers of the U. S. A. Medical Corps and 9 medical officers nominated by the Chinese Government. The remainder were medical officers of the R. A. F., R. A. M. C. and I. M. S. and cantonment antimalaria officers and assistants nominated by the War Department.

Much work has been done in regard to the testing of insecticides and mosquito repellents. An emulsifier for use with pyrethrum extract has been evolved, which renders the use of kerosene oil as a diluent unnecessary in the spray-killing of adult mosquitoes. A gum jelly containing pyrethrum extract has now been recommended for the army as a repellent in place of the greasy preparation formerly used.

Many tests have been carried out on sprayers both for spray-killing adult mosquitoes and for larvicidal work. Certain improvements have been introduced into the standard hand sprayer supplied to the army.

Laboratory researches have been restricted mainly to the preparation of antisera for the determination of the source of mosquito blood meals. The effect of certain drugs on monkey malaria has been investigated, and a number of tests have been carried out on stains for the diagnosis of malaria parasites.

Field researches in certain villages near Delhi with special reference to the spray-killing of adult mosquitoes, have been continued. The antimalaria operations in Delhi have again been supervised by the Institute staff. The occurrence of a regional malaria epidemic affecting Delhi Province has provided a rich field for investigation. In North Kanara, epidemiological observations have been carried out by a special research unit with its headquarters at Sirsi. A complete malaria survey is being conducted in this area.

The study of mosquito bionomics, especially of laboratory colonies of *Aedes aegypti* and *Anopheles stephensi*, has been continued, and the routine identification of mosquitoes and other insects of medical importance has been carried out as in former years.

Southern India branch (Coonoor).

(i) A large number of mosquito dissections have been carried out. *A. fluviatilis* has been incriminated as the vector in a malaria outbreak which occurred at an elevation of 5,000 feet.

(ii) In the course of infectivity experiments with *P. knowlesi*, 2 specimens of *Armigeres obturbans* became infected. Sporozoites from one of these injected into a *sinicus* monkey produced an infection which was successfully transmitted to a second monkey.

(iii) Thirtyone species of culicine mosquitoes, belonging to 12 sub-genera, have been identified, the larvae of 10 of which had not previously been recorded in India.

(iv) Four species of culicine have been infected with parasites of avian malaria, 1 with *P. gallinaceum* and 3 with *P. praecox*.

(v) The blood picture of fowls infected with *P. gallinaceum* has been studied with special reference to the effect of phenylhydrazine hydrochloride.

(vi) Observations have been carried out on the egg patterns of wild caught *A. fluviatilis*.

PROPOSALS FOR 1943–44.

(a) *Delhi.*

1. Systematic testing of insecticidal sprays and larvicides and spraying apparatus.

2. Testing of the therapeutic effects and toxic action of drugs as evidenced by their effect on animal malaria as occasion demands.

3. Intensive study of bionomics of mosquito colonies maintained in the laboratory.

4. Determination of food preferences of various species of mosquitoes by the precipitin test.

5. Attempts to increase the efficiency of the various antisera employed for precipitin tests.

6. Observations on the bionomics of *A. culicifacies*.

7. Epidemiological studies on malaria conditions in certain villages and coolie camps.

8. Testing of larvicides under field conditions.

(b) *Coonoor.*

1. Observations on the bionomics of *A. fluviatilis* and other mosquito species of the Nilgiris foothills.

2. Epidemiological studies in certain villages in the Nilgiris.

3. Investigations regarding the spray-killing of adult mosquitoes.

4. Experiments on mosquito malaria transmission.

5. Maintenance and study of strains of *P. knowlesi* and *P. gallinaceum* and of mosquito colonies.

ENQUIRY INTO THE ADULT HABITS OF A. VARUNA UNDER MAJOR R. SENIOR WHITE.

Field experiments were carried out on a forest in Khairagarh State, C. P. Collections of mosquitoes were made in a trap net at 2 a.m. and 6 a.m., and in a number of rooms. The ratio of *A. varuna* caught to *A. fluviatilis* was 1 : 12. *A. varuna* appears to rest exclusively in houses, whilst *A. fluviatilis* largely

rests out of doors. The infectivity rate in *A. varuna* is considerably lower than in *A. fluviatilis*.

Dissections of *A. varuna* carried out in the Vizagapatam area in special trap nets baited with men and buffaloes yielded a sporozoite rate of 0.02 per cent, as compared with 3.7 per cent from house collections in the Satpuras, and 1.1 per cent at the experimental site in Khairagarh State. Precipitin tests on 858 specimens from Vizagapatam tested at the Malaria Institute of India gave the following results :—

Caught in walled cattle-sheds, human positive	.. 4.9%
Caught in buffalo baited trap hut, human positive	.. 8.3%
Caught in open-sided cattle-shed, human positive	.. 27.4%
Caught in human baited trap hut, human positive	.. 58.5%

It is concluded that the Northern Circars Plains race is negligible as a vector of malaria under natural conditions.

This enquiry is now terminated.

Pharmacological enquiry at the Haffkine Institute under Dr. B. B. Dikshit.

(i) *Studies on synthetic antimalarial drugs*—2-Nl—Sulphanilamido-5-ethylthiazole was found to have an action on *P. knowlesi* infections in monkeys equal to that of sulphathiazole ; treatment with 1 gramme doses daily for 3 days effecting a radical cure. The toxicity of the drug is low and it is absorbed fairly quickly from the gastro-intestinal tract. Nl-Methyl sulphathiazole was also found to have some antimalarial activity, but was less effective than the above mentioned drug, and its toxicity was greater.

(ii) *Studies in malarial immunity in monkeys*—(a) Certain toxic substances present in splenic extracts of monkeys previously infected with malaria were removed, after which it was found that adequate doses of the extract could be administered to normal monkeys without ill effect. A certain degree of immunity was conferred on normal monkeys by the injection of such extracts. (b) The R-E system in certain monkeys was blocked by intravenous injection of Indian ink after splenectomy. It was found that if intravenous injections of Indian ink are given and the blood of such splenectomized immune animals replaced with normal blood to the extent of about 70 per cent or more, the monkeys succumb to infection showing that the immunity is broken. A uniform suspension of pure carbon standardized by a special biological method was found to be more suitable for this work than commercial Indian ink which is not satisfactory.

Proposals for 1943—44.

1. Investigation of possible substitutes for quinine : (a) synthetic drugs, (b) indigenous drugs.
2. Further studies on immunity in monkey malaria.
3. Studies on chemotherapy of plague.

Filariasis enquiry under Dr. Sundar Rao.

(i) *Field work*—In Damda village, Drug District, C. P., where the infections were exclusively *W. malayi*, there were 80 cases of elephantiasis of the limbs in a population of 3,628. There was no case of elephantiasis of the genitals, hydrocele or chyluria in this area. The microfilaria rate was 20 per cent.

Mansonioides annulifera, *A. culicifacies* and *C. fatigans* were collected. In Bassein, Thana District, Bombay Presidency, the infections were exclusively *W. bancrofti*. At Muzaffarpur and Khagaria (Monghyr District) in Bihar, the infection rates (also exclusively *bancrofti*) were 16 and 22 per cent respectively. In Ramnagar, Benares State, also only *bancrofti* infections were encountered.

(ii) A number of transfusion experiments were conducted to study the periodicity of microfilariae.

(iii) Preliminary experiments were carried out on rabbits in connection with the study of filarial allergen. The treatment of allergy in filarial patients by the administration of allergen has given satisfactory results.

(iv) A number of drugs have been tested out for the treatment of filarial infection. Of these Fantorin and Anthiomaline gave promising results, whilst M. & B 744 and a proprietary preparation made by a local chemist were found to be ineffective.

Proposals for 1943-44.

1. Work on the distribution of the two species of filarial parasites in different endemic areas in India.

2. Study on the life-history of filariae and their embryos.

3. Continuation of the investigation on filarial allergen and its relation to those of allied nematodes.

4. To continue the work on the treatment of filarial infection and filarial disease.

Filariasis enquiry under Dr. T. Bhaskara Menon.

The work was almost entirely confined to the study of *Conspiculum guindiensis* in the lizard *Calotes versicolor*. The pathology of lizard filariasis with special reference to the site of election of the worm and the migration of the developmental forms was studied. The various developmental forms have been fully described. Histopathological studies of lizard filariasis were also made.

This enquiry has now terminated.

Enquiry on the manufacture of pressure containers for spray-killing adult mosquitoes.

The object of the enquiry is to evolve a type of pressure container equipped with pressure gauge and valve, suitable for carrying about in the field. An apparatus designed to be charged at a pressure of about 150 lbs. per square inch from a compressor has already been constructed, and work is progressing on another designed for a pressure of 100 lbs. per square inch. The work is expected to be completed within the current financial year.

Proposals for new enquiries.

1. *Enquiry on the use of vegetable oils and extracts as mosquito larvicides under D. P. H., Madras.*

In view of the present shortage of mineral oils, it is proposed to conduct a series of experiments with vegetable oils and extracts in an endeavour to discover suitable substitutes for larvicidal purposes.

2. *Enquiry into suitable substitutes for quinine and other synthetic remedies used in the treatment of malaria under Dr. B. N. Ghosh.*

In view of the present shortage of antimalaria drugs, it is proposed to carry out an investigation on indigenous drugs reported to have antimalarial properties and to undertake similar researches in respect of the sulphonamide group of drugs.

3. *Enquiry on antimalaria control in the Tungabhadra project under D. P. H., Madras.*

It is proposed to carry out experiments to determine the range of flight of the vector species of Anopheles mosquitoes in the area commanded by the Tungabhadra irrigation project.

The Committee made the following recommendations :—

EXISTING ENQUIRIES.

1. *Malaria Institute of India (Research Section) and Southern India Branch.* The Committee approved the proposals put forward.

2. *Enquiry into the adult habits of A. varuna under Major Senior White.* The Committee noted that this enquiry has now terminated.

3. *Pharmacological enquiry at the Haffkine Institute under Dr. B. B. Dikshit.* The Committee recommended the continuance of this enquiry in so far as it concerned the investigation of antimalarial drugs and malarial immunity.

4. *Filariasis enquiry under Dr. Sundar Rao*—The Committee recommended the continuance of this enquiry.

5. *Filariasis enquiry under Dr. T. Bhaskara Menon*—The Committee noted that this enquiry has now terminated.

6. *Enquiry on the manufacture of pressure containers for spray-killing adult mosquitoes*—The Committee noted that the work on this enquiry is expected to be completed in the current financial year.

PROPOSALS FOR NEW ENQUIRIES.

1. Enquiry on the use of vegetable oils and extracts as mosquito larvicides under D. P. H., Madras.

2. Enquiry into suitable substitutes for quinine and other synthetic remedies used in the treatment of malaria under Dr. B. N. Ghosh.

3. Enquiry on antimalarial control in Tungabhadra project under D. P. H., Madras.

The Committee did not recommend the adoption of the proposals for any of these three new enquiries.

REPORT OF THE NUTRITION ADVISORY COMMITTEE.

The Committee met on the 7th December, 1942, and considered the following :—

1. Nutrition work in Provinces and States.

The Committee took note of reports on the work of nutrition officers in Bengal, Bihar, Hyderabad and the Punjab. The nutrition officers from Bihar and Hyderabad were present and gave an account of their work. The report on the work in the Punjab was presented by the Director of Public Health, Dr. A. H. Butt. The work which is being carried out in these provinces follows the lines which have been indicated in various previous reports of the Nutrition Advisory Committee. The desirability of including nutrition officers in the Health Department of other Provinces and States was once more emphasised by the Committee in its discussions.

2. The productions of shark liver oil in India.

Reports on the production of shark liver oil in Madras, Bombay and Baroda were considered. Shark liver oil is now being produced in considerable quantity to meet the requirements of Civil and Military Hospitals and Dispensaries, and the demand for the oil is on the increase. Questions relating to the testing of the oil and its keeping quality were briefly discussed by the Committee.

3. The food situation in India.

The Committee took note of the following recommendations made respectively by the Food Production Conference on the 6th April, 1942, and the Central Food Advisory Council on the 25th August, 1942.

(i) "Steps should be taken to encourage the use of 'undermilled' or home pounded rice in place of highly milled rice with the object of making existing supplies of rice go further and to take the opportunity afforded by the present food production drive to instruct the cultivator to secure for himself a more balanced diet."

(ii) "To meet the shortage of rice in the country as a whole, consequent on the cessation of Burma imports the Council decided to recommend to Provincial and State Governments in areas where rice was in short supply, to urge people to consume less of rice and more of millets with a view to tide over the shortage and also to popularise with the help of their agricultural staff and nutrition experts, a more balanced diet consisting of rice, vegetable and millets instead of a simple diet of rice."

These resolutions were critically discussed by the Committee, which recommended that the attention of the authorities concerned should be drawn to the desirability of making full use of modern knowledge of nutrition in formulating plans for dealing with the food situation, as has been done in England and other countries during the war. Abundant knowledge is available about minimum food requirements, the nutritive value of Indian foods and the diets normally consumed in various parts of India, which might prove of value in the present circumstances. Trained workers are available who can collect information about the state of nutrition of the population and how far it has been affected

by the food situation. When shortage of foods which are ordinarily consumed makes it necessary for the population to use alternative foods, nutrition experts can advise about the value of such alternative foods and help to popularise diets which are as satisfactory and well-balanced as circumstances permit.

4. Distribution of Health Bulletins nos. 23, 28 and 30.

The following information was supplied to the Committee :—

Health Bulletin No. 23. "The nutritive value of Indian foods and the planning of satisfactory diets." A third edition of 15,000 copies was printed in 1941, of which 9,666 copies remained unsold. 5,000 copies of an Urdu translation were printed in 1942. Of these 559 have been distributed free and 7 sold.

Health Bulletin No. 28. "Rice" was printed in June, 1940. Of the 5,000 copies printed, 4,120 have been disposed of.

Health Bulletin No. 30. "Food and Diet (written for children)" was issued in October, 1942. 5,000 copies have been printed. 1,237 copies have been distributed gratis and 434 sold.

5. Training course in nutrition in Coonoor.

The usual course was given in April–June, 1942, the class including 16 members. This was the 6th annual course.

6. Statistical control of diet surveys.

The Committee discussed the question of carrying out diet surveys with due regard to modern statistical technique in order to minimise sampling errors. It was pointed out that nutrition workers are today aware of the necessity of making use of statistical technique in their investigations, and of working in collaboration with statisticians. The difficulties of applying statistical methods of sampling in diet surveys, e.g. of ensuring that the samples of families surveyed were typical of the larger groups of which they form part, were discussed. It was pointed out that a careful scheme of investigation drawn up on statistical lines might be impracticable because it was usually necessary to work in areas in which collaboration and assistance from individuals in close touch with the population could be obtained, and to choose for survey families ready to cooperate in the investigation. The question of the duration of a family diet surveys was also discussed. In view of these and other recognised problems and difficulties in connection with the conducting of diet surveys, it was decided that a notice on the subject should be prepared for the 1943 meeting of the Nutrition Advisory Committee, in which rules for the guidance of those undertaking diet surveys should be suggested. The task of preparing this note was entrusted to Drs. Aykroyd, Mitra and Sankaran.

7. Existing and proposed enquiries.

The Committee recommended the continuation of the following enquiries :–

(a) Nutrition research under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, Coonoor, and the additional nutrition enquiry.

(b) Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(c) Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

It was noted that I. R. F. A. support of this enquiry will terminate in 1944.

(d) Studies on human nutrition under Dr. K. P. Basu at Dacca University, Dacca.

(e) Studies on the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

(f) Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

The Committee, while recommending the continuance of this enquiry, considered that the investigator should pay more attention to the determination of plasma proteins in healthy individuals in order to establish standards, and that he should concentrate on the study of plasma proteins in a limited number of biological conditions, instead of attempting to cover too wide a field. It was also suggested that the question of the regeneration of plasma proteins in blood donors would be an interesting and important subject for study.

(g) Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G.S. Medical College, Bombay.

(h) Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

The Committee expressed the view that the scheme of further work proposed was over-ambitious and that, for the time being, the worker in question should confine himself to work on the synthesis anf vitamin C, in relation to the effect of magnesium, in rabbits and rats. The identity of the indophenol-reducing substance in animal tissues with ascorbic acid should be proved beyond doubt by the use of ascorbic oxidase or by other means. Statistical analysis of results obtained was necesary.

(i) Enquiries into (i) tests of the biological value of soya bean protein (ii) growth tests with soya bean and (iii) human experiments with soya bean under Dr. K. P. Basu, Dr. V. N. Patwardhan and Dr. Bashir Ahmad.

The Committee recommended the continuance of this enquiry, in accordance with the report of soya bean Sub-Committee attached to this report.

The following enquiries will terminate in 1943, further support not being requested :—

(a) Enquiry into calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(b) Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal by Lieut.-colonel E. O'G. Kirwan, Professor of Ophthalmic Surgery, Medical College, Calcutta.

The following new enquiries were recommended.

(a) Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the University Chemical Laboratories, Lahore.

(b) Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra, Officer-in-Charge, Public Health Laboratories, Bankipur, Patna.

(c) Enquiry on the deterioration of vitamin A, in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore.

(d) Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The following enquiries were not recommended.

(i) Enquiry on serum phosphates under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

(ii) Studies on the body surface areas of Indian under Dr. D. M. Telang of the Grant Medical College, Bombay.

It was suggested that this application might be considered at a later date.

Professor N. M. Basu is discontinuing his enquiry into malnutrition of Bengali boys with reference to vitamins B & C deficiency and has put forward proposals for enquiries into the biological value of food proteins in relation to the regeneration of plasma proteins particularly serum albumin and the determination of vitamin A status of human and of the influence of trace elements on various chemical reactions in tissues and organs on different enzyme systems of the body and on the hormones produced by endocrine organs. The Committee was of the view that Professor Basu's proposals were of an over-ambitious nature.

REPORT OF SOYA BEAN SUB-COMMITTEE. DECEMBER 7th 1942.

I

The Soya bean Sub-Committee, at its first meeting in December, 1941, recommended a series of experiments in different laboratories designed to throw light on the nutritive value of soya bean in comparison with that of various common Indian pulses. This programme of research has been carried out in four laboratories under Drs. Ahmad, Aykroyd, K. P. Basu and Patwardhan respectively. Considerable progress has been made with the experiments and the experiments on the biological value of proteins obtained by the growth and balance-sheet methods will be complete by the end of March, 1943. They will then be submitted to the Medical Statistician. Vitamin tests of soya bean and other pulses have also been carried out.

The experiments on the effect of supplementing a poor rice diet by soya bean and other pulses respectively may not be complete by the end of the current financial year.

A human experiment along the lines suggested by the Committee has been initiated by Dr. Ahmad in Lahore. The Committee recommends that the organisation of the experiment should be discussed with the Medical Statistician.

II

It is recommended that the enquiry should be continued for another year with the following programme of research :—

(a) The experiments on the supplementary value of various pulses when added to the poor rice diet should be concluded.

(b) Two or three further varieties of soya-bean should be investigated by the methods originally laid down by the Sub-Committee. These should be varieties grown in India and may be obtained through the Director of Agriculture, Bengal.

(c) Certain American workers have reported that the heating of the soya bean to 120°C increases the biological value of its proteins. Experiments on the effects of heat (autoclaving, frying, parching) on the biological value of the proteins of soya-bean and other pulses should be undertaken. At the same time, the effect of such forms of heat on the vitamin content should be determined.

(d) *Human experiments*—The experiments on school children initiated by Dr. Ahmad should be continued with careful statistical control. Dr. K. P. Basu will carry out human metabolism experiments on the biological value of soya bean proteins in comparison with that of the proteins of other pulses. The Committee hopes that other participants in the enquiry may be able to carry out some experiments on human subjects.

The Committee is of the opinion that the enquiry will be completed in 1943-44. It hopes that in December, 1943 it will be in a position to give a definite reply to the question under consideration, viz. whether the nutritive value of soya bean, in comparison with that of common Indian pulses is such as to justify from the standpoint of nutrition, the encouragement of the production and consumption of soya bean on a wide scale in India.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on 7th December, 1942.

Plague enquiry under the Director, Haffkine Institute, Bombay

(a) *Chemotherapy and serum treatment of plague*—As a sufficient number of cases had not been obtained in the previous year to give evidence of statistical value on the relative efficacy of treatment of plague cases with sulphathiazole and sulphathiazole plus serum, the trials were continued in the present year. After eliminating certain cases from the series, 111 cases were left for comparison.

The mortality in the two groups was 19.6% and 20%. Taking only the cases showing septicaemia at the commencement of treatment the following figures were obtained :—

Treatment	Number of cases	Deaths	Case mortality per cent
Sulphathiazole ..	18	9	50.0
Sulphathiazole plus antiplague serum ..	25	8	32.0
	43	17	..
Previous controls ..	75	68	90.6

The Committee considered these findings from two points of view :—

(i) does the evidence obtained indicate that combined treatment is better than sulphathiazole alone ?

and (ii) under practical conditions of use in India do the results suggest the advisability of the continuance of further trials of combined treatment ?

In the opinion of Dr. Raja the figures so far obtained do not constitute a sufficient basis for statistical assessment of the relative value of the two treatments. In order to obtain a sufficient basis it would be necessary to carry out the treatment on at least 200 septicaemic cases, which would involve the treatment of 600 cases in all. It appears unlikely that this could be done under the present conditions. A sufficiently strong indication of a higher value of the combined treatment as compared with the treatment of sulphathiazole alone has not been given, which would support the desirablility of further comparative trials and, in addition, as the conditions under which plague cases can be treated in the field do not as a rule permit of adequate use of serum treatment it is unlikely that further investigation of that form of treatment would be fruitful from a practical point of view.

The Committee is of opinion that the portion of the grant for the production of serum should be discontinued.

(b) *Plague vaccine*—The Committee noted the studies which have been carried out on the production of *Past pestis* strains of different degrees of virulence and immunogenic value in relation to selection for the purpose of the manufacture of vaccine. It was noted that the protective dose of vaccines killed by different methods were much larger than those of the same avirulent strains when used as live vaccines. There was little difference in the protective dose of the vaccines killed by different methods, and no advantage was shown by other methods over the standard method of heating at 54°C for 15 minutes followed by the addition of 0.5% phenol.

A comparison was made of agar-grown and broth-grown vaccines prepared from the usual virulent strains by the following methods :—

1. Three days incubation at 28°C in 600 c.c. of casein hydrolysate broth producing a growth of 1000 million organisms per c.c., and

2. Suspension from 3 days culture on agar at 37°C with the same bacterial content.

The mouse protective doses of these were approximately the same. The toxicity of the two types was also of the same order and much less than in the case of standard Haffkine Vaccine.

The Committee considered it desirable that a quantitative determination should be made of the relative content of envelope and somatic antigens in the two types of vaccine and that further protection tests should be carried out in different species of laboratory animals.

(c) The serological studies on *Past pestis* strains were noted and it was considered essential that these should be continued in relation to vaccine production.

Continuance of the enquiry—The Committee recommend that this enquiry be continued during 1943-44 and that the grant asked for be allotted with the exception that a sum of Rs. 3,124 be deducted on account of pay of two animal men and the feeding charges of horses (Rs. 624+Rs. 2,500). The total allotment recommended is Rs. 19,352. The lines of work to be followed should be

(i) continuance of field work in chemotherapy of plague including trial of sulphadiazine, the use of which has been suggested by the National Research Council, Washington, who will undertake to supply the drug if required.

(ii) Continuance of the work on plague vaccine including the tests suggested above on the new broth vaccine produced by 3 days incubation at 28°c. in casein hydrolysate medium.

(iii) Continuance of the serological and other studies on vaccine strains.

Plague enquiry, Hosur Taluq, Madras.

The report on the above enquiry was explained by Dr. Adiseshan, Director of Public Health, Madras. It is considered that the work done constitutes a useful study of special epidemiological conditions. Certain points of interest have been brought out particularly in regard to changes in the prevalance of flea species which may be due to a new type of house construction which has been adopted in the area. The influence of this on future recurrence of plague should be investigated.

It is noted from the report that cyanogas 'G' fumigant which had to be used in place of cyanogas 'A' dust was much less effective probably on account of a lower evolution of HCN from this product which is in the form of larger grains.

It is suggested that the trials which were made of pyrethrum emulsion for the destruction of fleas should be extended in comparison with kerosene oil as a spray or kerosene oil emulsion to determine their relative value for disinfestation.

The continuance of the enquiry—The Committee recommend that a contribution of Rs. 2,500/- should again be made towards this enquiry in relation to the studies on epidemiological and preventive measures which form part of the programme.

INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

A. (*1*). *Volume XXXI (1941)—The Journal*—With the re-arrangement made in 1940 it was possible to make Vol. XXIX coincide with the calendar year and the usual 4 quarterly numbers were published. The average size for a number was 233 pages of literary and tabular matter and 4 plates, representing a decrease of 84 pages quarterly as compared with the previous volume. All the 4 numbers appeared in time.

(*2*). *The Memoirs*—One Memoir (No. 33 - Dec. 1941) - 'Anaemia in Pregnancy in Calcutta' by L. E. Napier and M. I. Neal Edwards - was published with this volume.

(*3*). *The net cost of Vol. XXIX*—(inclusive of Memoir No. 33) was Rs. 9,682.

B. (*1*). *Volume XXX (1942) - The Journal* - During the year which has elapsed since the last meeting the January, April and July numbers of the Journal have been published. Owing to the small amount of material received, insufficient material was available for the usual October number. Publication was accordingly delayed and the 4th number for the volume will appear about the beginning of December. The average size of 3 numbers so far published was 160 pages and 4 plates - this representing a further drop of 73 pages in the size of the numbers. The appearance of the July number was delayed for a month owing to certain corrections and additions which had to be made to an important paper on transmission of kala-azar, the early publication of which was desirable.

(*2*). *The Memoirs*—One Memoir (No. 34 - March, 1942) — 'A Comparative Nutritional Survey of various Indian Communities' by D. C. Wilson and E. M. Widdowson - appeared with this volume.

C. *Distribution*—

Indian subscribers	112	=198	
Foreign subscribers	86		
Exchange list	179	=301	(less 87 suspended owing to War= 214)
Free list	122		
Voucher copies to advertisers, file, regn :, make-up, police, specimen, propaganda, etc. ..	40 approx:		
Total ..	539	or 452	
Balance left with Publishers for sale ..	161	or 248	(actual now).
Total copies printed	700		

The Exchange and Free lists have each been increased by 1 over last year by addition of new addresses. There has been a fall of 3 in Indian and 24 in Foreign subscribers, which according to the Publishers is due to War conditions as the majority of those cancelled are for enemy countries. In addition, about 87 Foreign addresses on the distribution lists have also been suspended owing to the War. There has been an increase by 5 in voucher copies to advertisers. There has thus been *a total decrease of 107* in the distribution as compared with last year. A large number of publications are obtained in exchange for the I. J. M. R. for the I. R. F. A. Library, though some from enemy countries have been suspended.

D. *Distribution lists*—The distribution lists of the Journal were last revised in July 1938 and a new corrected alphabetical list showing exchanges has been printed and copies are available from the office of the Secretary for distribution to workers under the I. R. F. A.

E. *Number of copies printed*—From July 1940 it has been decided to print only 700 copies instead of 900, as this number is considered sufficient, according to former experience, to meet all demands. Volumes I to VIII are out of print.

F. *The cost of printing the Journal*—The following statement shows the cost of production during the last five years :—

Cost of printing the Journal.

Number.	XXIV–XXV 1937–38	XXV–XXVI 1938–39	XXVI–XXVII 1939–40	XXVII–XXVIII–XXIX, 1940–41	XXIX–XXX. 1941–42	
April ..	4,468	2,415	3,276	5,137	2,052	at 700 copies
July ..	4,907	4,744	4,212	3,932	3,531	
October ..	4,622	3,801	4,611	4,717	3,413	
January ..	4,572	3,652	3,640	3,571	2,531	
Totals ..	18,569	14,612	15,739	17,357	11,527	
Average pages	278	251	260	317	214	

Recoveries.

April ..	1,534	2,965	1,628	1,452	1,325
July ..	1,534	429	1,628	1,451	1,325
October ..	1,185	1,048	838	963	837
January ..	1,185	1,048	838	963	837
Totals ..	5,438	5,490	4,932	4,829	4,324

G. *Net cost of the Journal and Memoirs*— The following statement shows the net cost of the production of the Journal and Memoirs during the last five years :—

Journal.

April ..	2,934	—549	1,648	3,685	727
July ..	3,373	4,316	2,584	2,481	2,206
October ..	3,437	2,752	3,773	3,754	2,576
January ..	3,387	2,603	2,802	2,608	1,694
Totals ..	13,131	9,122	10,807	12,528	7,203

	No. 29	No. 30	No. 31	..	No. 33
	2,369	2,849	1,754	..	1,565
			No. 32	..	No. 34
			1,632		1,627
GRAND TOTALS ..	15,500	11,971	14,193	12,528	10,395

For convenience of accounting all receipts are credited to the Journal account and the figures given for the Memoirs are the actual cost of production.

The net cost during 1941-42 after deduction of receipts was Rs. 10,395. All Publishers' bills for 1941-42 were submitted in time to make payments within the financial year.

H. *Financial position for the year 1942-43*— The budget allotment for the year was Rs. 12,000 for the cost of 4 quarterly numbers of the Journal and Rs. 2,250 for any Memoir which might be published. The bills for the numbers already published have not as yet been received. The following is the estimated expenditure :—

	Estimated. Rs.
Vol. XXX, No. 2, April, 1942	2,000
Vol. XXX, No. 3, July, 1942	2,000
Vol. XXX, No. 4, October, 1942	2,000
(January 1943 not to be published)	nil
Total Rs. ..	6,000

Owing to the small amount of material being received, a 4th number will not be published before the end of the present financial year. The saving anticipated is Rs. 8,250.

J. *General*—Publication of the Journal has been continued on the lines laid down at the Conference of 1932 and the services of the members of the Editorial Board have been made full use of by referring to them all papers dealing with the subjects in regard to which they have been nominated. An addition to the Editorial Board has been made by appointment of Dr. C. G. Pandit as a member for Virus Diseases.

The Publishers have been able to continue the printing of the Journal on the same type of paper as formerly and to maintain a high standard of printing and presentation. The usual shade of green cover paper being no longer available, a grey-granite shade has been substituted from April 1941.

In Vol. XXX (January to October 1942) out of 72 articles published 27 were on nutrition, 11 on bacteriology, 12 on biochemistry, 8 on pharmacology, and 3 each on haematology and kala-azar.

K. *Budget estimate for 1943-44*—It is not anticipated that material will be available for more than two numbers of average size during 1943-44. The dates of appearance of the 2 numbers will be determined by the receipt

of papers and it will probably be possible to produce one number in May and a second about October. The following is the estimated cost of publication during the year allowing for 700 copies of 2 numbers and 1 Memoir which may possibly be received :—

	Rs.
2 half-yearly issued of the Journal @ Rs. 3,500* ..	7,000
1 Memoir	2,000
Total Rs. ..	9,000

*This has been kept at Rs. 3,500 instead of Rs. 3,000 in the past, because revenue from subscriptions and advertisements will now be considerably reduced.

In the event of it being possible to publish a 3rd number an additional grant may be asked for.

It is proposed to reduce the annual subscription from Rs. 16 to Rs. 8, while the cost of individual single numbers will remain as before.

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1942.

A. Indian Journal of Medical Research and Indian Medical Research Memoirs.

ACHARYA, B. N., NIYOGI, S. P., and PATWARDHAN, V. N.—Balanced Diets. Part III. The Effect of Parching on the Biological Value of the Proteins of some Cereals and Pulses (Vol. 30, No. 1).

BASU, K. P., BASAK, M. N., and DE, H. N.—Studies in Human Nutrition. Part IV. Availability of Calcium ingested in the Process of chewing Betel-leaves with Lime (Vol. 30, No. 2).

BASU, K. P., DE, H. N., and BASAK, M. N.—Studies in Human Nutrition. Part V. The Bones of Small Fish as a Source of Nutritionally Available Calcium and Phosphorus (Vol. 30, No. 3).

BHAVE, P. D., and BOPAIYA, M. S.—Diet Surveys and Investigations of Hæmoglobin Levels in Coorg (Vol. 30, No. 1).

BOSE, B. C., and MUKERJI, B.—On the Suitability of the Guinea-pig Method of Digitalis Assay in India (Vol. 30, No. 4).

CHOPRA, R. N., BOSE, B. C., GUPTA, J. C., and CHOPRA, I. C.—A Preliminary Study of the Pharmacological Action of the Glucoside of *Cerbera odollam* (Vol. 30, No. 1).

CHOPRA, R. N., BOSE, B. C., GUPTA, J. C., and CHOPRA, I. C.—Alkaloids of *Rauwolfia serpentina* : A Comparative Study of their Pharmacological Action and their Role in Experimental Hypertension (Vol. 30, No. 2).

CHOPRA, R. N., CHOPRA, G. S., and CHOPRA, I. C.—*Cannabis sativa* in Relation to Mental Diseases and Crime in India (Vol. 30, No. 1).

CHOPRA, R. N., GUPTA, J. C., BOSE, B. C., and CHOPRA, I. C.—A Preliminary Note on the Pharmacological Action of the Glucosides from *Paris polyphylla* Sm. (Vol. 30, No. 1).

DHARMENDRA—The Immunological Skin Tests in Leprosy. Part I. The Isolation of a Protein Antigen of *Mycobacterium leprœ* (Vol 30, No. 1).

DHARMENDRA and LOWE, J.—The Immunological Skin Tests in Leprosy. Part II. The Isolated Protein Antigen in Relation to the Classical Mitsuda Reaction and the Early Reaction to Lepromin (Vol 30, No. 1).

DHARMENDRA and LOWE, J.—The Immunological Skin Tests in Leprosy. Part III. The Isolated Protein Antigen in Relation to the Antigens used by other Workers (Vol. 30, No. 1).

DUTT, A. T., GHOSH, SUDHAMOY, and CHPORA, R. N.—The Chemical Investigation of the Gum Resin from *Balsamodendron mukul* Hook. (Vol. 30, No. 2).

KAMALA BHAGVAT, and NARASINGA RAO, K. K. P.—Vitamin C in Germinating Grains (Vol. 30, No. 4).

KAMALA BHAGVAT, and NARASINGA RAO, K.K.P.—Vitamin C Content of Dry Bengal Gram (*Cicer arietinum*) (Vol. 30, No. 4).

LAL, R. B., and DAS GUPTA, A. C.—Investigations into the Epidemiology of Epidemic Dropsy. Part XV. Incidence by Season (Vol. 30, No. 1).

MANGALIK, V. S., GOEL, M. P., and MANGALAIK, HEMLATA.—Studies in Gastric Acidity in Indians with Alcohol Test-Meal. (Vol. 30, No. 2).

MUKERJI, B., DUTTA, N. K., and GANGULY, S. C.—Studies on some Dextro-Rotatory Hydrocupreidine Derivatives. Part III. Comparative Effects on *Paramœcia caudatum* (Vol. 30, No. 2).

PANJA, G.—A New Method of Isolation of Vibrios from Cholera Stool. (Vol. 30, No. 3).

PATWARDHAN, V. N., and CHITRE, R. G.—Studies in Calcium and Phosphorus Metabolism. Part V. The alleged Influence of Vitamin D on the Absorption of Calcium from the Intestine : Experiments with Albino Rats. (Vol. 30, No. 1).

RANGANATHAN, S.—The Vitamin C Content of some Dehydrated Vegetables and Fruits, and of Fruit Juice Preparations. (Vol. 30, No. 4).

RANGANATHAN, S.—Vitamin C Content of Tender Walnut (*Juglans regia*) (Vol. 30, No. 4).

RAO, M. V. RADHAKRISHNA—Intestinal Changes in Monkeys fed on Poor Rice Diets. (Vol. 30, No. 2).

RAO, M. V. RADHAKRISHNA, and RAO, M. NARASIMHA—Hæmatological Investigations in South India. Part VI. The Red Cell Diameter in Healthy Europeans and Indians. (Vol. 30, No. 1).

RAO, S. SUNDAR— Observations on Filariasis in Lakhipur and Binakandy Tea-Gardens (Cachar District, Lower Assam). (Vol. 30, No. 2).

READ, W. D. B., PANDIT, S. R., and DAS, P. C.—Action of *V. cholerae* and El Tor Type Strains on Goat's Red Corpuscles. (Vol. 30, No. 2).

RUDRA, M. N., and ROY, S. K.—On Serum Phosphatase. Part I. The Serum Phosphatase in Pulmonary Tuberculosis and the Effect of Ingestion of Vitamin C upon it. (Vol. 30, Nol. 4).

SARKAR, B. B., MAITRA, S. R., and GHOSH, B. N.—The Effect of Neurotoxin, Hæmolysin and Choline Esterase Isolated from Cobra Venom on Heart, Blood Pressure and Respiration. (Vol. 30, No. 3).

SEAL, S. C.—A Note on the Production of *Clostridium perfringens* (Type A) Toxin in a Modified Liver and Veal Digest Medium. (Vol. 30, No. 2).

SHOURIE, K. L.—Dental Caries in Children in Madras City in Relation to Economic and Nutritional Status. (Vol. 30, No. 4).

SHOURIE, K. L., and SUNDARARAJAN, A. R.—The Effect of Milling on the Vitamin B_1 (Thiamine) and Nicotinic Acid Content of Indian Wheat Products. (Vol. 30, No. 1).

SWAMINATH, C. S., SHORTT, H. E., and ANDERSON, L. A. P.—Transmission of Indian Kala-azar to Man by the Bites of *Phlebotomus argentipes*, Ann. and Brun. (Vol. 30, No. 3).

SWAMINATHAN, M.—A Fluorimetric Method for the Estimation of Riboflavin in Foodstuffs. (Vol. 30, No. 1).

SWAMINATHAN, M.—Flourimetric Estimation of Riboflavin in Urine. (Vol. 30, No. 1).

SWAMINATHAN, M.—Flourimetric Assay of Riboflavin in the Urine and Tissues of Rats. (Vol. 30, No. 1).

SWAMINATHAN, M.—An Improved Method for the Estimation of Vitamin B_1 in Foods by the Thiochrome Reaction. (Vol. 30, No. 2).

SWAMINATHAN, M.—A Simple Procedure for Estimating Nicotinic Acid in Biological Materials using the Cyanogen Bromide-Aniline Reagent. (Vol. 30, No. 3).

SWAMINATHAN, M.—Note on the Vitamin B_1, Riboflavin and Nicotinic Acid Content of Dried Yeast. (Vol. 30, No. 3).

SWAMINATHAN, M.—The Effect of Washing and Cooking on the Vitamin B_1 Content of Raw and Parboiled Milled Rice. (Vol. 30, No. 3).

SWAMINATHAN, M.—The Application of Cyanogen Bromide Test to a Study of the Metabolism of Nicotinic Acid in Rabbits. (Vol. 30, No. 4).

TAYLOR, J.—EDITORIAL NOTE on "The Transmission of Kala-azar" (Vol. 30, No. 3).

WILSON, D. C., and WIDDOWSON, E. M.—A Comparative Nutritional Survey of various Indian Communities. (*Memoir* No. 34, March, 1942).

B. *Journal of the Malaria Institute of India.*

COVELL, G., and PRITAM SINGH.—Malaria in the coastal belt of Orissa. (Vol. IV, No. 4).

C. *List of papers published by workers under the I. R. F. A. in Journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

AYKROYD, W. R.—Infantile Beri-beri. (I. M. G., 1942, 77, 2, p. 97).

AYKROYD, W. R.—Stability of Ascorbic Acid in Dehydrated Vegetables. (Nature, in press).

AYKROYD, W. R.—Public Health and Nutrition. (I. M. G. 1942, 77, 9, p. 549).

AYKROYD, W. R.—The Food Situation in India. (Indian Jour. Social Work, 1942, 3, 2, p. 148)

BANERJEE, S., and GUHA, B. C.—Intradermal test as an index of vitamin C nutrition. Parts III, IV & V. (Ann. Biochem. Exp. Med. Vol. II, No. 2).

BASU, K. P., and NATH, H. P.—Role of phosphorus, flavin and hormones in the utilisation of proteins (Ann. Biochem. Exp. Med. 2, 63, 1942).

Biological value of the proteins of papaya and lady's finger (Ann. Biochem. Exp. Med., 2, 71, 1942).

BRAGANCA, B., GHOSH N. C. and SAHA, K. C.—Vitamin A and D contents of certain fish liver oils. (Ann. Biochem. Exp. Med. Vol. II, No. 2).

COCHRANE, R. G.—Prognosis and Treatment of Leprosy with special reference to its Pathology. (Journal of the Christian Medical Association of India, Vol. XVII, No. 3, May 1942).

COVELL, G.—The public health aspect of malaria control. (I. M. G. Vol. LXXVII, No. 11).

COVELL, G.—Note on economy in the use of antimalaria drugs (I. M. G. Vol. LXXVII, No. 12).

DAS GUPTA, C. R.—Indian Made Liver Extracts in the Treatment of Macrocytic Anaemia. (I. M. G., Vol. LXXVII, No. 12).

DEY, N. C., and MAPLESTONE, P. A.—Tinia imbrication in India (I. M. G. Vol. LXXVII, January, 1942).

DHARMENDRA—Studies of the Lepromin Test (9) A bacillary antigen standardised by weight. (Lep. in India, Vol. XIV, No. 4).

DHARMENDRA & LOWE, J.—Studies of the Lepromin Test. (6) Results of the Mitsuda Test in cases of leprosy of different clinical types. (Lep. in Ind. Vol. XIV, No. 1).

DHARMENDRA, LOWE, J. and MUKHERJI, N.—Studies of the Lepromin Test. (7) Variations in the results of the Mitsuda test observed in cases of leprosy of the neuro-macular type. (Lep. in India, Vol. XIV, No. 3).

DHARMENDRA, LOWE, J., and MUKHERJI, N.—Studies of the Lepromin Test. (8) Attempts to increase the reaction to lepromin in cases of leprosy by repeated testing. (Lep. in India, Vol. XIV, No. 3).

GUHATHAKURTA, S. R.—Tuberculin Test—its Value in the early diagnosis of Pulmonary Tuberculosis, Trans. Med. Coll. Re. Union, Vol. V. 1942).

GUPTA, J. C., BOSE, B. C., GANGULI, S. C., and CHOPRA, I. C.—A preliminary study of the Riedal-Walker Coefficient values of certain Indigenous Essential Oil. (I. M. G., Vol. LXXVII, No. 4).

LAHIRI, M. N.—A study on Leptospirosis in Bombay City. (I. M. G.).

LOWE, J.—Leprosy and Blood Groups (Lep. in India, Vol. XIV, No. 1.)

LOWE, J.—Separation of children from infectious parents and others (Lep. in India, Vol. XIV, No. 4).

LOWE, J.—Comments on the history of leprosy (Ind. Med. Gaz. Vol. LXXVII, No. 11).

NAPIER, L. EVERARD.—Blood and Plasma Transfusion. (I. M. G., Vol. LXXVII, No. 3).

PANJA, G., MALIK, K. S., and PAUL, B. M.—Examination of cholera vomit. (*Ind. Med. Gaz.* Vol. LXXVII, No. 6., p. 347).

PANJA, G., MALIK, K.S., PAUL, B. M. and GHOSE, S. K.—Treatment of cholera with pyrogen free saline. (*Ind. Med. Gaz.*, Vol. LXXVII, No. 5, p. 282).

RAJAGOPAL, K.—A Sample of Shark Liver Oil Unsusually Rich in Vitamin A. (Current Science, 1942, 11, 2, p. 52).

SARMA, P.S., and BHAGVAT, K.—Accumulation of Pyruvic Acid in Rice Moth Larvae. (*Corcyra cephalonica Staint*) fed on a Vitamin B_1 Deficient Diet. (Current Science, 1942, 11, 8, p. 331.)

SARMA, P.S., and BHAGVAT, K.—The Synthesis of Vitamin C by Rice Moth Larvae (*Corcyra cephalonica Staint*) (Current Science, 1942, 11, 10, p. 394).

SEN, P.K.—Behaviour of Contralateral Lung in Ambulant cases under Unilateral Pneumothorax Treatment, I. M. G., Special Tuberculosis Number, October, 1942.

SEN, P. K.—The place of Collapse Theory in Pulmonary Tuberculosis. The Med. Coll. Magazine, Vol. X, No. 1, June, 1942.

Early Diagnosis in Pulmonary Tuberculosis, Trans. Med. Coll. Re. Union, Vol. V. 1942).

SWAMINATHAN, M.—Riboflavin and its Role in Nutrition. (I.M.G., 1942, in press).

TELANG, D. M.—Physical Efficiency Test of Schneider on Bombay Medical Students. (I. M. G. Vol. LXXVII, No. 1).

UKIL, A. C.—Early Diagnosis and Treatment of Intestinal Tuberculosis, I. M. G., special Tuberculosis number, October, 1942.)

VERMA, O. P.—Further Experience of the Treatment of Superficial Keratitis with Riboflavin. (I. M. G., 1942, 77, 8, p. 471).

VERMA, O. P.—Partial Degeneration of the Optic Nerve Associated with vitamin Deficiency. (I. M. G., 1942, in press).

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 8th December, 1942.

	Enquiries.	Budget allotments. Rs.
1.	Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta	4,184
2.	Enquiry to determine the endemic foci of cholera in certain parts of Bengal and Madras Presidencies under Dr. K. C. K. E. Raja, Offg. Deputy Public Health Commissioner with the Government of India, New Delhi	2,900
3.	Malaria Institute of India, Research Section, Delhi and Kasauli	32,434
4.	Southern India Branch of the Research Section of the Malaria Institute of India, Coonoor	11,642
5.	Nutrition Research Laboratories, I. R. F. A., Coonoor ..	84,739
6.	Additional nutrition enquiry, (a) Assistant Director, Nutrition Research Laboratories, I. R. F. A., Coonoor, (b) three field workers and (c) the enquiry on beri beri in Coconada. (*The cost to be met from the balance of Rs. 30,000/- annual grant sanctioned by the Governing Body over a period of five years, out of the special ten lakhs grant made by Government for antimalaria and nutrition work*)	23,847
7.	Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	5,635
8.	Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	3,208
9.	Enquiry entitled "Studies on human nutrition with Indian diets" under Dr. K. P. Basu at Dacca University ..	4,983
10.	Enquiry entitled "Studies on the metabolism of vitamin A and carotene" under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta	2,885
11.	Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	2,009
12.	Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay	4,060
13.	Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna	1,831

	Enquiries.	Budget allotments. Rs.
14.	Enquiries into (i) tests of the biological value of soya bean protein, (ii) growth tests with soya bean and (iii) human experiments with soya bean under Dr. W. R. Aykroyd, Dr. K. P. Basu, Dr. V. N. Patwardhan and Dr. Bashir Ahmad	5,650
15.	Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta	6,808
16.	Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras ..	2,190
17.	Plague researches under the Director, Haffkine Institute, Parel, Bombay	19,352
18.	Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore	2,474
19.	Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay	2,703
20.	Indigenous drugs enquiry under Dr. J. C. Gupta, Professor of Pharmacology, School of Tropical Medicine, Calcutta ..	10,098
21.	Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra at the Drug Research Laboratory, Jammu, Kashmir ..	3,592
22.	Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay	4,480
23.	Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	4,804
24.	Enquiry into the causes of infant morbidity and mortality under Dr. Jean M. Orkney, W.M.S., Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi ..	1,200
25.	Enquiry entitled "Studies in tests of physical fitness in Indians" under Dr. D. M. Telang at the Grant Medical College, Bombay	1,928
26.	Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta	5,876
27.	Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta	5,172
28.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	3,896
29.	Kala-azar enquiry under the Director, Pasteur Institute, Shillong	12,490
30.	Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay	3,734

	Enquiries.	Budget allotments. Rs.
31.	Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta ..	6,600
32.	Enquiry into the occurrence of ascites in Hyderabad under Professor S. W. Hardikar at the Osmania Medical College Hyderabad (Deccan)	2,286
33.	Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	2,896
34.	Enquiry into the isolation of atropa from dhatura under Dr. J. N. Ray at the Delhi University Laboratories, Delhi ..	3,116
35.	Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the University Chemical Laboratories, Lahore	2,300
36.	Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra, Officer-in-Charge, Public Health Laboratories, Bankipur, Patna	1,904
37.	Enquiry on the determination of vitamin A in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore	2,200
38.	Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	1,680
39.	Enquiry on the statistical evaluation of anti-cholera inoculation in the Madras Presidency under the Director of Public Health, Madras	2,000
	Grants.	
(*i*)	Administration charges	37,645
(*ii*)	Contribution towards the cost of two professorships at the School of Tropical Medicine, Calcutta	27,204
(*iii*)	Contribution towards the cost of plague enquiry in the Salem District under the Government of Madras ..	2,500
(*iv*)	Contribution to the Imperial Institute of Entomology, London (£. 100/-)	1,350
(*v*)	Contribution to the Bureau of Hygiene and Tropical Diseases, London (£. 150/-)	2,025

	Grants.	Budget allotments. Rs.
(*vi*)	Contribution towards the cost of the Asst. Public Health Commissioners with the Government of India and the Statistical Bureau in the office of the Public Health Commissioner with the Government of India	6,650
(*vii*)	Four research fellowships of the value of Rs. 150/- p.m. each	7,200
(*viii*)	Indian Journal of Medical Research and Memoirs (*a*) Publication charges of the Journal .. 7,000 (*b*) Publication charges of the Memoirs .. 2,000 (*c*) Editor's office 4,500	13,500
(*ix*)	I. R. F. A. Library at the Central Research Institute, Kasauli	1,712
(*x*)	Pay (including special pay, overseas pay and allowances) and contributions towards passage, leave salary and pension or contributory provident fund of the officers of the Medical Research Department under the Association	1,50,000
(*xi*)	Travelling allowance and certain contingent expenses for Medical Research Department Officers employed under the I. R. F. A. and attached to Central or Provincial laboratories	2,000
(*xii*)	Pay and travelling allowance on transfer of the three workers in the Junior Research Cadre	20,040
(*xiii*)	Clerical assistance at the School of Tropical Medicine, Calcutta	3,150
(*xiv*)	Clerical assistance to the Director, All-India Institute of Hygiene and Public Health, Calcutta	1,308
(*xv*)	Travelling allowance of subordinate I. R. F. A. workers on transfer from one post to another	1,500
(*xvi*)	I. R. F. A. contribution and interest towards the Contributory Provident Fund	13,000
(*xvii*)	Miscellaneous unforeseen expenditure	10,000
(*xviii*)	Provision for imprest advance for enquiries	7,425

JUN 7 1944

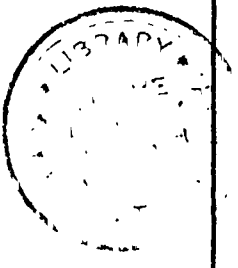
REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1943.



Issued under the authority of the Governing Body,
Indian Research Fund Association,
New Delhi,
India.

Price per copy One Rupee.

Obtainable from the Secretary, Governing Body, Indian Research Fund Association, Secretariat,
NEW DELHI (India).

With the compliments of

Lieut.-Colonel E. Cotter, C.I.E.,

I.M.S., Secretary, Governing Body

and Scientific Advisory Board,

Indian Research Fund Association.

NEW DELHI
INDIA.

CONTENTS.

Page.

I. Composition of the Board i
II. Composition of Advisory Committees ii
III. Meetings held during the year iii
IV. Researches carried out during the year .. .. .. 1—109

CHOLERA.

Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta .. .. 1—7

Enquiry to determine the endemic foci of cholera in certain parts of Bengal and Madras Presidencies under Rao Bahadur Dr. K. C. K. E. Raja, Offg. Deputy Public Health Commissioner with the Government of India, New Delhi .. .. 7—9

Enquiry on the statistical evaluation of anti-cholera inoculation in the Madras Presidency under the Director, King Institute, Guindy, Madras and the Director of Public Health, Madras .. 9—21

MALARIA.

Malaria Institute of India (Research Section), Delhi .. .. 22—23

NUTRITION.

Nutrition researches under Dr. W. R. Aykroyd at the I. R. F. A. Nutrition Research Laboratories, Coonoor .. .. 24—27

Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science & Technology, Calcutta .. .. 27—28

Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science & Technology, Calcutta .. 28—31

Studies in human nutrition with Indian diets under Dr. K. P. Basu at Dacca University, Dacca .. .. .. 31—33

Studies in the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta .. .. 33—34

Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay .. .. 34—37

Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay .. .. .. 37—39

Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna .. .. 39—40

Page.

Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean (3) human experiments with soya bean under Dr. K. P. Basu, at the Dacca University, Dacca.. 41—42

Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay .. .. 42—43

Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. Bashir Ahmad at the Unversity Chemical Laboratories, Lahore .. .. 43—44

Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the University Chemical Laboratories, Lahore .. .. 44—45

Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipur, Patna .. .. 45—46

Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore .. .. .. 46—47

Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay 47

Enquiry to organise research into the best methods of treating starvation cases under the Director, All-India Institute of Hygiene and Public Health, Calcutta .. .. .. 47—51

LEPROSY.

Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta .. .. 52—56

Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras .. .. 56—62

PLAGUE.

Plague researches under the Director, Haffkine Institute, Bombay. 63—64

Plague enquiry in the Salem District under the Director of Public Health, Madras 65

PHARMACOLOGY.

Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore .. .. .. 66—67

Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay .. .. .. 67—68

Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta .. .. .. 68—70

Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S., at the Drug Research Laboratory, Jammu, Kashmir .. .. 70—71

Page.

Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay .. .. 71—72

MATERNAL MORTALITY.

Enquiry into the causes of infant mortality and morbidity under Dr. Jean Orkney, W.M.S., Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi .. .. 73—78

OTHER RESEARCHES.

Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta .. .. 79—82

Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta .. .. .. 82—84

Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay .. .. 84—85

Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta .. .. 85—88

Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras .. .. 88—90

Kala-azar enquiry under the Director, Pasteur Institute, Shillong 90—93

Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay .. 93—95

Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta .. 95—96

Enquiry into the occurrence of ascites in Hyderabad State under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan .. .. 96—101

Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta .. 102–103

Enquiry into the isolation of atropa from dhatura under Dr. J. N. Ray at the Delhi University Laboratories, Delhi .. 103–106

Leptospirosis enquiry under Dr. M. N. Lahiri at the Haffkine Institute, Bombay 107–109

V. Reports of Advisory Committees on

Malaria 110–112

Nutrition 113–134

Plague 135–136

VI. Indian Journal of Medical Research and Indian Medical Research Memoirs 137–139

VII. Miscellaneous papers issued under the auspices of the Indian Research Fund Association 140–143

VIII. Programme of researches and miscellaneous grants recommended for the financial year 1944-45 144–147

The Scientific Advisory Board consisted of the following :—

Chairman :

Lieut.-General Sir Gordon Jolly, K.C.I.E, K.H.S., I.M.S., Director-General, Indian Medical Service (1st January to 3rd October, 1943).

Major-General J. B. Hance, C.I.E., O.B.E., K.H.S., I.M.S., Director-General, Indian Medical Service, New Delhi. (4th October, to 31st December, 1943).

Members :

Lieut.-Colonel E. Cotter, C.I.E., I.M.S., Public Health Commissioner with the Government of India, New Delhi. *Secretary.*

Major-General Sir John Taylor, C.I.E., D.S.O., I.M.S., Director, Central Research Institute, Kasauli.

Dr. L. E. Napier, C.I.E., F.R.C.P., Director, School of Tropical Medicine, Calcutta. (1st January to 3rd March, 1943.)

Dr. B. M. Das Gupta, Director, School of Tropical Medicine, Calcutta. (4th March to 31st December, 1943)

Lieut.-Colonel S. S. Sokhey, I.M.S., Director, Haffkine Institute, Parel, Bombay.

Dr. C. G. Pandit, O.B.E., M.B.B.S., Ph.D., D.P.H., D.T.M., Director, King Institute, Guindy, Madras.

Brigadier G. Covell, C.I.E., I.M.S., Director, Malaria Institute of India, Delhi.

Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S., Director, Drug Research Laboratory, Jammu, Kashmir.

Dr. V. R. Khanolkar, M.D., B.Sc., Director of Laboratories, Tata Memorial Hospital, Parel, Bombay.

Dr. G. Sankaran, M.B.B.S., Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Co-opted members :

Dr. J. B. Grant, M.D., Director, All-India Institute of Hygiene and Public Health, Calcutta (for enquiries based at the All-India Institute of Hygiene and Public Health, Calcutta).

Dr. W. R. Aykroyd, C.B.E., M.D., Director, Nutrition Research Laboratories, Coonoor (for the subject of nutrition).

Composition of Advisory Committees for the year 1944.

1. *Malaria.*

Brigadier G. COVELL (*Chairman*).
Lieut.-Colonel M. K. AFRIDI.
Dr. ANANTASWAMI RAO.
Rai Bahadur Dr. A. C. BANERJEA.
Brevet-Colonel Sir RAM NATH CHOPRA.
Dr. B. M. DAS GUPTA.
Lieut.-Colonel H. W. MULLIGAN.
Dr. D. K. VISWANATHAN.
Major JASWANT SINGH (*Secretary*).

2. *Nutrition.*

Lieut.-Colonel E. COTTER (*Chairman*).
Professor S. P. AGHARKAR.
Dr. K. P. BASU.
Khan Bahadur Dr. A. H. BUTT.
Sir THEODORE GREGORY.
Dr. B. C. GUHA.
Dr. V. N. PATWARDHAN.
Dr. G. SANKARAN.
Mr. J. SARGENT.
Mr. H. R. STEWART.
Mr. F. WARE.
Dr. W. R. AYKROYD. (*Secretary*).

3. *Plague.*

Lieut.-Colonel S. S. SOKHEY (*Chairman*).
Dr. R. ADISESHAN.
Lieut.-Colonel L. A. P. ANDERSON.
Dr. V. R. KHANOLKAR.
Dr. C. G. PANDIT.
Major-General Sir JOHN TAYLOR (*Secretary*).

The Board held two Meetings during the year, the first on the 13th December and the second on the 14th and 15th December, 1943.

At its meeting on the 13th December, 1943, the Board confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers and also decided on the co-option of experts for its meeting on the 14th and 15th December, 1943. On these dates the Board considered in detail the programme of research for the year 1944-45. The programme of researches recommended by the Board is given on pages 144-147 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1943.

The researches, which were carried out during the year under report, and which were recommended by the Board at its meeting on the 8th December, 1942, were approved by the Governing Body of the Association at their meeting held in the Imperial Secretariat, New Delhi, on the 6th March, 1943.

CHOLERA.

1. Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta.

SULPHANILYLGUANIDINE.

(i) *Less mortality with treatment* : This drug has now been administered to a total of 251 clinical cholera cases up-to-date. During the current year the number of sulphanilylguanidine treated cases was 174 out of which there were 3 or 1.72% deaths. Out of the 174 control cases there were 11 or 6.32% deaths.

(ii) *Method of administration* : The drug was administered along with other routine treatment as soon as the patients were admitted into the hospital. To allay vomiting the drug was either mixed with iced water or plain water followed by ice to suck. It was given to them to drink in small quantities.

(iii) *Dose* : 5 gms. of the drug were given immediately on admission and 2.3 gms. every 4 hours after, for 24 hours, making a total of 20 gms.

(iv) *Early passage of urine* : Out of 174 sulphanilylguanidine treated cases 76 were admitted with suppression of urine. Of these 42 passed urine within 24 hours, 21 within 48 hours and 13 within 72 hours.

Out of 174 control cases 72 were admitted with suppression of urine. Of these 23 passed urine within 24 hours, 26 within 48 hours and 23 within 72 hours.

(v) *Uraemia* : 9 cases who developed uraemia inspite of the routine treatment were later on treated with sulphanilylguanidine; only one died.

(vi) *Saline infusion less required* : Average amount of saline solution required for each of the sulphanilylguanidine treated cases was 3.4 pints whereas for the control cases it was 5.4 pints.

(vii) *In vitro experiments* : A toxic strain of V. cholerae was grown in nutrient broth and later on sulphanilylguanidine was added to it. The same strain was also grown in a nutrient broth to which the drug was previously added. There were growths in both media, showng that the drug had neither bacteriocidal nor bacteriostatic effects.

(viii) *In vivo experiment* : Guinea-pigs were infected orally with broth cultures of a toxic strain and later on the drug was administered by mouth. The results show a definite curative effect of the drug.

Sulphasuxidine.

This drug was administered to 59 cases and there were 3 or 5.2% deaths whereas in 59 control cases there were also 3 or 5.2% deaths. Results are inconclusive and more cases are being tried.

Pyrogen free water.

Since it was difficult to obtain fresh distilled water in large quantities for the preparation of saline solution, the following method was adopted to prepare pyrogen free water by chemical treatment.

2000 c.c. of tap water or pyronic distilled water (previously known to contain pyrogen) was acidulated with 0.4 c.c. of strong commercial sulphuric acid and treated with 4 c.c. of 0.4 per cent solution of potassium permanganate. This was boiled for 2 hours. The colour of the water was observed during the process of boiling. More potassium permanganate was added if the colour disappeared completely. There should be at least a faint trace of colour till the end of boiling. It was then covered and allowed to cool.

It can, at this stage, be preserved with advantage for a pretty long time. No organism would grow in this acid water and produce the pyrogen. Further any organic matter that might have accidentally contaminated it would be oxidised by the potassium permanganate already in it. Hence it must always be observed that there is a faint trace of colour present in it before use. It would always be safer to add more potassium permanganate solution to it if there be no colour and boil again. In this process it does not require so much of sterility to preserve it as is required for distilled water. Pyrogen grows very rapidly in water if it is not preserved aseptically. So the chemically treated water is much more advantageous than the distilled water for storing purposes.

At the time of use the water was filtered through filter paper and a few (5-10) drops of hydrogen peroxide were added to it and then shaken. The colour disappeared within a minute or two. It was then slightly heated to remove the excess of hydrogen peroxide. When cooled, pH was adjusted by gradual addition of caustic soda solutions to 7.1 with the help of Hellige indicators and comparator. This was done as follows: Three solutions (1 in 100, 1 in 1,000 and 1 in 10,000) of caustic soda were kept ready in stock. 5 c.c. of water was then taken in a test tube and 0.125 c.c. of phenol red was added to it. The colour became yellow. To this tube caustic solution was added from the stock solutions by means of a pipette till the colour matched with the colour of 7.1 in the Hellige comparator or with a standard tube made by us. The standard tube contains a solution of distilled water and phenol red and adjusted to 7.1 by caustic soda solution. The quantity of caustic soda solution required in 5 c.c. was then measured. From this the quantity of caustic solution to be required for the whole amount was calculated out and added. When thoroughly mixed up pH was again tested in 5 c.c. of water after the final addition of the caustic solution. Pyrogen free water was thus prepared.

The effects of pyrogenic reaction following intravenous injections of hypertonic saline solutions prepared in tap water, distilled water and pyrogen free water in cholera cases and rabbits are given below :

In rabbits.

Serial No.	Sodium chloride solvant.	No. of rabbits used.	Average normal temperature of these animals.	Average normal variation.	Average temperature at the time of injection.	Average maximum temperature after the injection within 2 hours.	Average variation between the temperature before and the highest after injection.	Actual variation due to injection.	Average pH of the saline.
1.	Tap water.	10	102.47	0.26	102.36	104.3	1.94	1.68	8.5
2.	Distilled water.	14	103	0.4	103	103.4	0.4	0	7.1
3.	Pyrogen free water.	10	102.4	0.56	102.6	102.7	0.1	0	7.1

In cholera cases.

Serial No.	Sodium chloride solvant.	No. of cases.	Average temperature before injection.	Average maximum temperature after injection within 2 hours.	Average variation.	Rigor.	pH of the solution.
1.	Tap water.	7	96	103	7	+	8.5
2.	Distilled water.	6	97.4	98.4	1	—	7
3.	Pyrogen free water.	20	98.4	99.8	1.4	—	7.1

From the above findings it appears that pyrogen free water can be prepared by treating water with acid potassium permanganate as by the process of distillation.

PYROGENIC REACTION FOLLOWING INTRAVENOUS INJECTION OF SALINE.

In order to render saline solution used in cholera cases non-pyrogenic, hypertonic solution was prepared in distilled water and it was observed that it did not produce any rigor or rise of temperature in cholera cases. It was concluded from these results that these reactions were due to the pyrogenic substances in water. Later on alkaline saline solution which is required extensively in the treatment of cholera was prepared in distilled water and injected intravenously into patients. There was rigor and rise of temperature though to a less extent in almost all of them. These findings suggested the planning of the following experiments on rabbits and cholera cases.

The following saline solutions were injected intravenously in 10 c.c. amounts into healthy rabbits and in 3 pints doses in cholera cases and their pyrogenic effects were studied under the following heads :

(a) Rectal temperature was recorded before and after the injections every 15 minutes for 2 hours. The variation of temperature was the difference between the temperatures immediately before and the highest after the injection during this period of 2 hours. (b) The presence of pyrogenic substance was demonstrated and estimated by potassium permanganate and ammon. oxalate titration in these saline solutions. (c) pH of these saline solutions was determined by Hellige indicators.

The following saline solutions were used in the experiment :—

(1) Hypertonic saline solution— 140 gr. of sodium chloride in 1 pint of tap water.

(2) Hypertonic saline solution made with the above ingredients in freshly prepared distilled water.

(3) Alkaline normal saline solution— 90 gr. of sodium chloride and 90 gr. of sodium bicarbonate in 1 pint of tap water.

(4) Alkaline normal saline solution made with the above ingredients in freshly prepared distilled water.

(5) As a contrast to alkaline normal saline solution in distilled water an acid normal saline solution was prepared with 90 gr. of sodium chloride in freshly prepared distilled water and pH was adjusted to 5.2 with dilute sulphuric acid. (Freshly prepared distilled water was used to prepare dilute sulphuric acid.) Merck's sodium chloride and sodium bicarbonate were used in these experiments.

(6) Hypertonic saline solution— 140 grs. of sodium chloride in 1 pint of tap water—pH adjusted to 7.1.

(7) Hypertonic saline solution— 140 grs. of sodium chloride in 1 pint of tap water already treated with acid potassium permanganate to oxidise the organic matter present in it and pH adjusted to 7.1.

The results of experiments on rabbits are shown in table 1 and of cholera cases in table II.

Table I.

Serial No.	Materials.	No. of animals used.	Average normal temperature of these animals.	Average normal variation.	Average temperature at the time of injection.	Average maximum temperature after injection within 24 hours.	Average variaton between the temperature before and the highest after the injection.	Actual variation due to injection.	Average pH of the injectable substances.	Average amount of oxygen consumed to oxidise the organic matter present in 100 c.c of water.
1.	Hypertonic saline solution in tap water	10	102.47	0.26	102.36	104.3	1.94	1.68	8.5	0.25 mgm
2.	Hypertonic saline solution in distilled water.	14	103	0.4	103	103.4	0.4	0	7.1	nil.
3.	Alkaline normal saline solution in tap water.	10	102.23	0.36	102.1	104.4	2.34	1.98	10.2	0.25 mgm
4.	Alkaline normal saline solution in distilled water.	15	103.05	0.33	102.95	104.03	1.08	0.75	9.1	nil.
5.	Acid normal saline solution in distilled water.	12	102.34	0.12	102.4	103.27	0.87	0.75	5.2	nil.
6.	Hypertonic saline in tap water adjusted to 7.1.	10	101.93	0.24	102.1	103.8	0.98	0.74	7.1	0.25 mgm
7.	Hypertonic saline in tap water treated with potassium permanganate.	10	102.44	0.56	102.62	102.76	0.14	?	7.1	nil.

Table II.

Serial No.	Materials.	No. of cases.	Average temperature before injection.	Average maximum temperature after injection.	Average variation of temperature.	Rigor.	Hydrogen ion concentration.	Average amount of oxygen consumed to oxidise the organic matter present in 100 c.c. of water.
1.	Hypertonic saline solution in tap water.	7	96°F	103°	7°	+	8.5	0.25 mgm
2.	Hypertonic saline solution in distilled water.	6	97.4°	98.4°	1°	—	7	nil.
3.	Alkaline normal saline solution in tap water.	6	96.5°	102.8°	6.3°	+	9.5	0.25 mgm
4.	Alkaline normal saline solution in distilled water.	33	96.9°	100.6°	3.7°	+	9.1	nil.
6.	Hypertonic saline in tap water adjusted to 7.1	7	98.5°	102°	3.5°	+	7.1	0.25 mgm
7.	Hypertonic saline in tap water treated with potassium permanganate.	20	98.4°	99.8°	1.4°	—	7.1	nil.

It was observed from the results of the experiments that :—

1. Hypertonic saline solution made in fresh distilled water and chemically treated water which did not contain any oxidisable matter and whose pH was about 7.1 did not produce rigor or appreciable rise of temperature in patients or rabbits.

2. Hypertonic and alkaline saline solutions made in tap water were alkaline in reaction and contained a large amount of oxidisable matter. They produced severe reactions both in rabbits and men.

3. Alkaline saline solutions made in freshly distilled water and chemically treated water did not contain any oxidisable matter but were alkaline in reaction. They produced a mild reaction.

4. Hypertonic saline solution in tap water whose pH was adjusted to 7.1 but contained the same amount of oxidisable matter produced a mild reaction.

5. Hypertonic saline made in distilled water whose pH was adjusted to 5.2 and contained no oxidisable matter did also produce a mild reaction in rabbits only.

Thus it can be concluded that the reactions following intravenous injections of saline solutions are due to pyrogen (a bacterial product), an oxidisable matter and higher or lower hydrogen ion concentration of the solution.

pH OF THE BLOOD IN CHOLERA CASES

The pH of the blood of cholera cases is less alkaline than normal and it was found that it increases with the injection of alkaline saline solution even though uraemic condition still appeared in later stages. The figures are very small and no definite conclusion can yet be drawn. The work is being continued. *Blood sugar* in cholera cases is definitely low : attempts are being made to reduce the uraemia by introducing glucose into all the saline solutions used. The work is in progress.

2. Enquiry to determine the endemic foci of cholera in certain parts of Bengal and Madras Presidencies under Rao Bahadur Dr. K. C. K. E. Raja, Offg. Deputy Public Health Commissioner with the Government of India, New Delhi.

The purpose of this enquiry is that of devising suitable methods (and if such be possible easy methods) for the purpose of differentiating local areas in respect of their cholera incidence, in order to determine whether the disease exists in such areas in endemic or epidemic form. Endemicity can be determined only in terms of available information for attacks and deaths due to the disease for a long period of time. For an extensive period only figures for deaths are available. These figures are published by health authorities for weeks and months, so that a finer distribution of cholera mortality in relation to time is not available for our investigation. This is a definite handicap because the finer the classification the greater will be the accuracy of any method used for investigating the problem of endemicity.

It seems reasonable to assume that, in a truly endemic area, the intervals showing absence of infection should be rare and purely of a random nature

In a method devised by Neyman and Pearson, which may be described as the method of 'interval' analysis, it has been shown that intervals between events, either in space or time, if they are of a random nature, should be distributed according to the exponential law. This method was used on the cholera mortality figures of a number of places but was found to be unsatisfactory, mainly because the data at our disposal are grouped too coarsely to satisfy the conditions of this method. Another contributory reason is that the assumption of randomness in respect of the intervals between events on which the method is based, may not be wholly true in respect of cholera. For this disease there is a specific seasonal periodicity in many places (cholera appears year after year during particular months) while some investigators (Russell and Sundararajan) have also attempted to show a long term periodicity of five or six years, at the end of which outbreak takes place on an intensive scale.

In view of the fact that the material for study at our disposal is lacking in fineness it is recognised that any method that may be evolved can result only in the demarcation of endemic and non-endemic areas on very general lines. However even such a demarcation should be of value from the point of view of the public health worker, because it will enable him to recognise those areas where efforts for an improvement of environmental sanitation and for other measures directed against cholera should be concentrated. The method that is being presented in the succeeding paragraphs is one which lacks fineness. Still it is sufficiently simple to be applied by public health workers on a wide scale.

An endemic area should have normally a much lower percentage of weeks or months free from the disease than a non-endemic area. The average percentage of weeks or months free from recorded cholera deaths in these areas is therefore a useful criterion. However, it has but limited value. We have to take into account not only the number of weeks or months free from the disease but also the length of the periods showing absence of the disease. For this the mean of the distribution of such intervals constitutes a useful criterion.

In order to apply these criteria a number of places showing only small intervals of absence of cholera as well as others with long periods of absence were taken. The former can be considered as examples of endemic areas and the latter as non-endemic areas. Certain other places where the distribution of cholera deaths were of an intermediate nature were also examined. On the results of this examination the following criteria were defined :—

Criterion (1) *Percentage of free months.*

Endemic areas	less than or equal to 30.
Intermediate	between 30 and 50.
Non-endemic	50 and over.

Criterion (2) *Mean length (in months) of gaps between outbreaks.*

Endemic	less than or equal to 2.5.
Intermediate	between 2.5 and 4.
Non-endemic	equal to or greater than 4.

The frequency distribution of breaks in infection were graphed. These graphs will be shown to the Scientific Advisory Board. All the curves show an exponential trend, which is very pronounced in the curves for endemic areas.

In endemic places there are likely to be very few long breaks in the continuity of infection and so the curves for these areas are steep, while the curves for the non-endemic areas are rather flat. This is as it should be. The curves suggest an equation ($y=Ae^{-Bx}$) where x is the duration of break and y is its frequency.

The constants, A and B, have been fitted by the least square method, to the straight lines obtained by taking logarithms of the figures on both sides of the equation. It was seen that the values of B were greater in endemic areas. The value of B may prove to be an additional aid in differentiating an endemic from a non-endemic area. However, it cannot be considered as a separate criterion from criterion No. (2) because, on theoretical grounds, B is only the reciprocal of the mean in an infinite population of intervals of absence of infection, from which our distributions of such intervals for separate local areas are taken to be samples. Thus the value of B in respect of any area is highly correlated with criterion No. 2 for that area and it serves to reinforce the inference we draw from No. (2).

The work carried on these lines have covered the following :—

Madras Presidency :—26 centres (rural and urban) covering Tanjore, Trichinopoly and South Arcot districts. The result of the analysis shows 23 of them as " non-endemic ", 2 " intermediate " and 1 " endemic ".

Bengal Presidency :—180 places (rural and urban). Out of them 55 are " non-endemic ", 45 " intermediate " and 80 " endemic areas ". They cover the districts of Mymensingh, Dacca, Jessore, Faridpur, Noakhali, Tippera, Chittagong, Bakarganj, Khulna, Birbhum and 24 Parganas. Here all local areas, urban and rural, have been examined. In 12 other districts a certain number of centres have been dealt with. These were taken up in a piecemeal fashion because we were, at the time, investigating well defined endemic and non-endemic areas for the purpose of evolving our method of establishing endemicity. These districts are, Burdwan, Bankura, Midnapur, Howrah, Nadia, Bogra, Rajshahi, Murshidabad, Rangpur, Dinajpur, Jalpaiguri and Darjeeling.

3. Enquiry on the statistical evaluation of anti-cholera inoculation in the Madras Presidency under the Director, King Institute, Guindy, Madras and the Director of Public Health, Madras.

Apart from Russell's survey on limited statistical material on the comparative value of anti-cholera inoculation and oral administration of bilivaccine for personal prophylaxis, no survey based on sufficiently large statistical material appears to have been done to assess the value of anti-cholera inoculation in the prevention and control of cholera. Further, the vaccine used now (containing both Inaba and Ogawa antigens) is probably somewhat different from the vaccine used in Russell's survey. It was thought that the very large number of inoculations done during the unprecedentedly severe epidemic of cholera in Madras Presidency in 1942 would afford exceptionally good opportunity for the conduct of an investigation to assess the value of anticholera inoculations in conferring personal and mass immunity. The suggestion made on this behalf to the Scientific Advisory Board was readily accepted by them ; approval of the Madras Government was also secured. With the financial assistance from the Madras Government and a subvention from the Indian Research Fund Associaton, the present enquiry was undertaken.

As a basic idea of the epidemiology of cholera in Madras Presidency and of the system of public health administration with special reference to notification, prevention and control of epidemics would be helpful in the consideration of the subject, these are dealt in paragraphs 2 to 7 below :

2. The Public Health Administration is more or less parallel to the system of Revenue Administration. The system of Revenue Administration is briefly as follows : the executive chief for each of the 24 districts in the Presidency is the Collector. Every district is composed of a number of taluks—8 to 12 in a district. The taluk revenue official is the Tehsildar. Each of the taluks comprises several villages—ranging anywhere from 50 to 200. Besides the main portion, every village contains several suburbs which are designated in this Province as " hamlets ". These hamlets may contain either more or less self contained units of population or specific population groups; e.g., weavers, farmers, depressed class labourers etc., who have communal lives of their own.

Every district contains a certain number of municipalities ranging from one to six. There are altogether 82 municipal towns in the Presidency ; the civic administration of these towns is governed by the Municipal Acts.

3. As regards Public Health Administration, the great majority of the 82 municipalities have Health Officers (Government officers) and subordinate staffs (Municipal employees). For the rural areas which form the bulk of the district both as regards size and population, there is a Health Officer and an Assistant Health Officer for each district. Generally, for each taluk (called a range), there is a Health Inspector who is a qualified Sanitary Inspector. The Health Officers, Assistant Health Officers and Health Inspectors belong to the Provincial Service. Besides the above staff, there are in every district, vaccinators, licence-inspectors, etc., who are servants of Local Bodies. Many of these subordinates are qualified Sanitary Inspectors and can be diverted for public health work other than their primary duties. The whole of the public health subordinate staff in each unit—municipal or district rural—is under the administrative control of the Health Officer.

4. The legislative enactment which contains most of the provision regarding notification and control of epidemic diseases, is the Madras Public Health Act of 1939.

(a) As regards notification of infectious diseases, all doctors must notify cases of infectious diseases to the health authority or village headman in the case of rural areas ; a similar obligation rests on the householders when a direction to that effect is issued by the Collector of the district. Whilst a small proportion of the cases is notified by the doctors and the public, the larger proportion is detected by the health staff in towns and the village headmen in rural areas : as regards the latter, the health staffs also discovered an appreciable number of missed cases. The village headman sends a copy of the epidemic report to the Tahsildar and another copy directly to the Health Inspector in order to enable the latter to take immediate preventive and control measures. The Tahsildar sends daily to the Director of Public Health, District Health Officer and other specified officers in the district a consolidated return for the infected villages in his jurisdiction. In the case of municipalities, these daily returns are sent to the Director of Public Health by the Municipal Health Officers or Executive Authorities. The returns for the Province are consolidated in the Director of Public Health's Office in the epidemic section under the immediate control of an Assistant Director. There are four other Assistant Directors for other subjects.

(b) Under the provisions contained in the Madras Public Health Act, Government have powers to appoint Special Health Officers in areas affected by or threatened with epidemics ; the Director of Public Health also possesses powers in connection with prevention and control of epidemics ; in addition to giving general directions to Local Bodies on the above subject, he may assign to any local area the public health personnel from other areas whether these belong to provincial or local services. Health Officers have inherent powers under the Public Health Act to appoint additional staffs, concentrate in an area members of the staff from other places in the Health Officer's jurisdiction, purchase medicines and equipment, carry out disinfection measures, etc. ; the last of these powers is conferred on Health Inspectors also. When the Collector of the district notifies an area to be affected by or threatened with an epidemic or any of the scheduled diseases of which cholera is one, the Health Officer gets additional powers, viz., control of movements of population and goods, disinfection, closure of markets, enforcement of inoculations and vaccinations compulsorily and other like measures. It may also be stated in this connection that rules to supplement these measures have been framed in several instances by the Government and these rules have the force of law.

It may also be stated here that, under the above Act, Municipal authorities are statutorily bound to provide for public health administration a minimum of thirty per cent (30%) of their total income and District Boards a minimum of 12½ per cent of their income.

5. According to Russell, who made an elaborate statistical study of the subject, the epidemiology of cholera in Madras Presidency is closely correlated to monsoons. According to the conclusions arrived at by him, the Presidency is divisible into three districts geographical groups as regards the seasonal incidence in general of cholera prevalence.

(a) North-East and West Coast districts which derive most of the rainfall from South-West monsoon : Seasonal incidence of cholera is in June to September.

(b) South-East coastal districts which get the bulk of the rain from North-East Monsoon cholera incidence is in the colder months of the year, October to January.

(c) Ceded and Central districts which derive varying amounts of rain from both the monsoons : there are two distinct seasonal waves, corresponding to the two monsoons.

Besides this, correlation of the yearly seasonal cholera incidence to the monsoons, there is a seven-yearly periodicity when there is a marked exacarbation in the incidence of the disease.

6. The last periodicity of cholera in Madras Presidency was in 1935-36, when practically every district was affected and 70,000 deaths were recorded.

The present widespread " periodic " wave of the epidemic commenced late in the first quarter of 1942 in Bellary in the Ceded districts, where there were semi-famine conditions at that time. The infection continued during the monsoon months and there were two peaks in the course of the South-West and North-East Monsoons (group C in previous para). The infection was imported from here into south-eastern coastal districts (group b above). It was rather extraordinary that the districts in group (a) virtually escaped infection in 1942. In one of these districts however, viz. Malabar (West Coast District), there was a devastating cholera epidemic this year. Also infection has just

started in one of the North-East districts (West Godavari District). Further, there has been a recrudescence of the epidemic in the South-East districts especially, Tinnevelly, the southernmost district in the Madras Presidency.

7. The epidemic of 1942 prevailed in 18 out of the 24 districts of the Province; in some areas, there was more than one outbreak during the period. The epidemic was widespread and severe; there were about 52,000 deaths during the year. The provisions of the Public Health Act were fully availed of in control work. All available resources in man and material were pooled and concentrated wherever needed. Besides additional staff, the services of paid and subsidized medical personnel were fully utilized in control work. Inoculation was declared compulsory but the voluntary response was generally very satisfactory and the compulsory provisions were enforced only in rare instances. The number of inoculations done during the year was 44 millions.

8. Owing to administrative difficulties, it was obviously impossible to extend the scope of the enquiry to all the affected districts. It was therefore confined to South Arcot district which comprises 3 municipalities and 2,300 villages. The municipalities were not included in the survey as the conditions therein are different from those in the rural areas. Out of 2,300 villages in the district with a population of 2.5 millions, 1,605 villages were affected by cholera, the total incidence of cases and deaths being about 30,000 and 16,400 respectively. The population exposed to risk was about 1.02 millions, and of these 0.76 millions (75 per cent of the population at risks) were inoculated.

9. The suggestion was made that the most useful data would be those secured on an experimental basis by carrying out anticipatory inoculations in certain villages and leaving certain other villages under more or less identical conditions wholly unprotected. But this was not possible for several reasons. In the first place, it is only on the actual occurrence of cholera in the village or in the immediate neighbourhood that the public volunteer or could be persuaded to be inoculated. Secondly, when there is a demand for inoculation, it would be impracticable for administrative reasons, to inoculate one section of the population and leave the other section uninoculated simply for the sake of an experiment. Thirdly, on account of the widespread nature of the epidemic, the available field staff and vaccine supplies were just sufficient to meet the needs of the areas actually affected. It may however be stated in this connection that, in several instances, villages experienced more than one outbreak of cholera in the present epidemic. The inoculations done in the first epidemic in a village amount virtually to anticipatory inoculations with respect to the subsequent outbreaks in the same village.

10. According to the rules now in force, the village headman of every village should give prompt information to the health staff of the occurrence of every cholera case in the village. He should also maintain a register wherein particulars of the cases with respect to name, age, sex, date of attack and date of death in fatal cases should be recorded. The Health Inspector is required to maintain among other records, an inoculation register showing dates of inoculation, and the name, sex, age and address of the persons inoculated. As the village cholera registers do not contain information whether the cases had been previously inoculated, the Collectors of the districts were requested to direct village headmen to enquire and record in the register in respect of every case whether the person was previously inoculated or not and if inoculated, the date of inoculation. A village headman is intimately acquainted with the people of the village. The particulars recorded in the cholera register are therefore

generally accurate. These entries were further checked by the Health Inspectors by reference to the inoculation registers.

11. The data were collected on cards specially devised and printed for the purpose. The form prescribed contains all the particulars considered necessary for the enquiry and could be easily filled in from the registers referred to above. According to the distribution of infection in the village, the whole village or affected hamlet was taken as a unit and the particulars were recorded on a separate card. Every card received from the health staff was scrutinized and discrepancies and omissions rectified before it was included in the compilation.

12. The anti-cholera vaccine used in the investigation was manufactured in the King Institute of Preventive Medicine. It comprises Inaba and Ogawa antigens. The vaccine is in conformity with the prescribed standards.

13. The great proportion of the inoculations done by qualified Sanitary Inspectors trained in inoculation work. Only a small proportion of inoculations was performed by qualified medical men.

In the greater generality of instances, the vaccine was administered in a single dose, as this alone is possible in mass inoculation campaigns necessary in epidemic emergencies. In the case of adults, the dosage was 1 c.c., half this dose was given in the case of children.

14. The survey has been carried out in close collaboration with Dr. C. G. Pandit and Dr. K V. Venkataraman, Director and Assistant Director of King Institute, Guindy. The statistical analysis has been done by Mr. V. K. Parthasarathy, the Statistician in charge of the Vital Statistics Section in the office of the Director of Public Health, Madras.

15. The total number of cards received was 2,670. It has been possible to scrutinise and analyse the larger proportion of cards relating to only one district viz., South Arcot. The present preliminary report deals with the study of the data relating to 1,283 villages in the above district.

16 The following is a brief summary of the scope of the enquiry :

(a) Whether anti-cholera inoculation confers immunity against cholera and, if it does, the time taken for its development from the date of inoculation. At present, on the presumption that inoculation confers immunity, an International standard of six days has been prescribed as the period for the development of the immunity. The data required for determining the above are (i) the relative incidence of cholera in the inoculated and uninoculated groups of the population exposed to risk and (ii) the incidence of cholera in the inoculated group of population exposed to risk classified according to the occurrence of the cases on the days succeeding the date of inoculation and a similar distribution of incidence according to data in respect of the uninoculated also.

(b) The duration of the immunity, if any, conferred by inoculation : This could be determined from the relative incidence of cholera among the uninoculated and inoculated persons in the populations at risk of villages which experienced more than one outbreak of disease. (If figures by varying periods of intervals are available).

(c) The influence, if any, of herd immunity on the duration of the epidemic and on the occurrence of subsequent outbreaks within a reasonable interval, this could be determined from the data in (a) and (b) above. The former has not yet been worked out.

(d) Relative, sex and age incidence of cholera and any other subject of importance that may arise in the analysis of the data. These have not yet been taken up for consideration.

As regards (a), (b) and (c) above, although it has not been possible to subject to analysis the data contained in all the survey cards that have been received, the statistical material that has been analysed is sufficiently large for the purpose of the present preliminary report.

17. For the purpose of the present study, the following definitions have been adopted :

(i) No prophylactic vaccine gives instantaneous immunity. Varying periods are required for the development of a sufficiently high level of immunity. The period of six days prescribed in the International Quarantine Regulations for considering as protected a person inoculated with anti-cholera vaccine has been adopted for the differentiation of inoculated persons from those " protected " by inoculation.

(ii) In the case of villages which experienced more than one outbreak of cholera, the epidemics were considered as separate and distinct if the interval between the successive outbreaks was 30 days and above. This is in order to avoid any fallacies that might arise due to missed or sub-clinical cases.

Alternative classifications with two weeks', three weeks' and four weeks' intervals were also tried in 163 villages in Villupuram range; this does not make any material difference (vide Annexure I).

(iii) The duration of an epidemic in a place is defined as the number of days, both inclusive, elapsing from the date of the first to the date of report of the last case in the outbreak.

(iv) If the distribution of cases of cholera was more or less general in a village, the census population of the village was considered as the population at risk. If the incidence of cases was restricted to one or more hamlets only, the population of these hamlets as determined by rough enumeration were taken as the population at risk.

18. WHETHER INOCULATION CONFERS IMMUNITY AGAINST CHOLERA.

(a) The population of the 1,283 villages the data of which have been included in the present study is 1,334,016. Of this population 392,115 are protected as per definition in para supra. The rest of the population comprises uninoculated persons as also persons who were inoculated but are not classified as " protected " as they are within the 6 day period following the date of inoculation ; the population in these two groups has not been differentiated. This will be done however in the further stages of the study in order to elimnate any possible fallacies that might result from the inclusion of persons who might have been partially protected within 6 days of inoculation. The incidence of cholera in the protected and unprotected groups in all villages taking the whole experience into consideration may be compared. In the great majority of cases, the inoculations were done on different dates during the course of epidemic. Thus the ratio of the protected to the unprotected population (including the partially immunized) varied from day to day. The weighted mean of protected persons in each village has been taken as the protected, the weights used being days of exposure. The rest of the population was taken as unprotected which includes the partially immunised population.

		No. attacked.	Incidence rate per mille of population.
Protected	392,115	571	1.5
Unprotected	941,901	16,198	17.1

The incidence of cholera in the unprotected group is 11.4 times as much as in the protected. Is this difference significant ? Applying the X^{-2} test to the fourfold table :

	Not attacked.	Attacked.	Total.
Protected	391,544	571	392,115
Unprotected	925,793	16,108	941,901
Total	1,317,337	16,679	1,334,016

the value of X^{-2} is 5478.7 and of P is less than .0016. The difference in rates is therefore significant.

(b) Reference has been made in para 9 above that in the case of the villages which experienced more than one outbreak of cholera, the inoculation done in the first outbreak as also those done six days prior to the commencement of the second outbreak, may be treated as anticipatory inoculations with respect to the second outbreak. 387 villages out of the total of 1,283 villages comprised in the present study had more than one outbreak. The total population of these 387 villages is 435,553. Of this, 1,40,745 comprise the protected population as per definition. The rest are *uninoculated*. The fallacy that might result from the inclusion of partially immunised persons referred to in the previous sub-para is thus eliminated herein. The incidence of cholera in these two groups is as follows :—

		No. attacked.	Incidence rate per mille of population.
Protected	140,745	149	1.06
Uninoculated	294,808	3,394	11.51

Here again, the incidence rate in the uninoculated group is 10.9 times the rate in the protected group. This closely corresponds to the figure in the previous para. Is this difference in rates significant ? Applying the X^{-2} test to the fourfold table :

	Not attacked.	Attacked.	Total.
Protected	140,596	149	140,745
Uninoculated	291,414	3,394	294,808
Total	432,010	3,543	435,553

the value of X^{-2} is $1286 \div 4$ and of P is less than 0.0016. The difference in rates is significant.

19. (a) Time interval after inoculation for the development of immunity.

The time interval taken for establishing immunity may be examined by comparing the incidence rates in the inoculated group day by day commencing from the date of inoculation. It should however be stated that the phase of epidemic at which inoculation was done has not been taken into account. The following table shows the incidence among the inoculated day by day in all the villages under investigation.

Total population of all villages .. 1,334,016

Total number inoculated in all villages .. 516,230

Period.	No. of attacks among inoculated.	Incidence rates per mille of inoculated.
Same day	198	0.38
1st day	402	0.78
2nd ,,	414	0.80
3rd ,,	406	0.79
4th ,,	298	0.58
5th ,,	224	0.44
6th ,,	141	0.27
7th ,,	90	0.18
8th ,,	62	0.12
9th ,,	38	0.07
10th ,,	30	0.06
11th and after	334	0.65
Total	2,637	

The difference in the incidence rates between the successive days may be tested for significance by X^{-2} test, viz. the table for the 2nd day and 3rd day is as follows :

	Attacked.	Not attacked.	Total
2nd day	214	515,216	515,630
3rd day	406	514,810	515,216
Total	820	1,030,026	1,030,846

The value of X^{-2} is 0.07 and of P=0.79. The difference is not significant.

The values of X^{-2} and of P for the difference in rates between successive days are given below : The value of P 0.05 is taken as the level of significance.

Periods.	Difference in incidence rates per mille of population.	Value of X^{-2}	value of P.		Significances.
Day of inoculation and following day.	0.40	68.8	(Less than)	0.01	Significant
1st day and 2nd	0.02	0.18	0.67		Not significant.
2nd „ „ 3rd	0.01	0.07	0.73		do.
3rd „ „ 4th	0.21	17.74	(Less than)	0.0016	significant.
4th „ „ 5th	0.14	10.5	do.	0.0016	do.
5th „ „ 6th	0.17	17.2	do.	0.0016	do.
6th „ „ 7th	0.09	10.9	do.	0.0016	do.
7th „ „ 8th	0.06	5.2	do.	0.02	do.
8th „ „ 9th	0.05	5.8	do.	0.016	do.
9th „ „ 10th	0.01	0.9	do.	0.34	Not significant.

(Note.—The comparison of incidence rates on the day of inoculation and the first day is not strictly valid, as the period of exposure on the ' inoculation day ' is not one of complete 24 hours, the inoculations being done at different hours of the day).

It would seem from the above that, on each of the three days following the date of inoculation, the rate of incidence of cholera among the inoculated shows little variation ; this rate shows a progressive decline from the fourth day onwards and the minimum is reached on the 10th day after inoculation. The marked increase in the rate on the 11th and subsequent days is probably associated with the problem of the duration of immunity and requires further investigation ; the cases in this group include incidence of cholera upto a period of about six months in the inoculated.

20. It will be seen from paragraph 18 that the uninoculated persons in a population at risk are susceptible to an extent of over 10 times in getting cholera as compared with the inoculated persons in the same population. The case fatality rates from cholera among the inoculated persons classified according to the occurrence of the cases on the several days following inoculation, are given in the following table :

Day after inoculation.	No. of cases.	No. of deaths.	Case fatality rate.
(1)	(2)	(3)	(4)
Same day of inoculation	198	108	54.5
1st day	402	188	46.8
2nd „	414	208	50.0
3rd „	406	197	48.5
4th „	298	125	41.9
5th „	224	111	49.6
6th „	141	66	46.8
7th „	90	41	45.6
8th „	62	25	40.3
9th „	38	20	52.6
10th „	30	9	30.0
11th „ and above	334	134	40.1
Total	2,637	1,232	546.7

The general case mortality rate among the uninoculated persons in the epidemic was nearly 63.9 per cent. It would seem from the above table that although the inoculated persons have far less chance of getting cholera than the uninoculated benefit to a similar degree is not conferred as regards survival after disease.

21. Out of 1,283 villages, 896 experienced single outbreaks of cholera whilst the remaining 387 had two or more outbreaks. If it is assumed that the only variable factor during the period in the case of these two groups is the difference in the proportion of inoculated populations, this would indicate the influence of herd immunity in governing the occurrence of the second outbreak. Figures relating to these are given below :

	Population.	Number inoculated at the end of 1st outbreak.	Rate per cent.
Villages with 1 outbreak	839,131	318,890	38.0
Villages with 2 outbreaks	494,885	140,745	28.4

Is the difference in the rates (0.096) significant ?

The best estimate of P=0.34 and Q=0.66.

The standard error of the difference is given by

$$= \sqrt{Pq\,(1/n_1 \text{ plus } 1/n_2)}$$

$$= \sqrt{.34 \times 0.66\,(1/839131 \text{ plus } 1/494885)}$$

$$= .0008.$$

$$\therefore \frac{\text{Difference in rates}}{\text{Standard error}} = \frac{.096}{.0008} = 113 \text{ nearly.}$$

The difference in rates is therefore significant.

22. The data of 1,283 villages affected by cholera in South Arcot district in 1942 have been analysed and studied. The following conclusions are indicated :

(1) Among the populations at risk, the group of uninoculated population and persons who had been inoculated but were within the six-day period after the date of inoculation seem to be susceptible to an extent over ten times in getting cholera as compared with inoculated populations after the sixth day of the inoculation. The mortality rates from cholera among the two groups of populations do not however show advantages to the same degree as the inoculated.

(b) 387 out of the 1,283 affected villages experienced more than one outbreak of cholera in the present epidemic at intervals of 30 days and over between the successive outbreaks. If the population group of 140,745 inoculated in the first outbreak and prior to six days before the date of the second outbreak is considered as anticipatory inoculations with respect to the second outbreak, the incidence rate of cholera in the above group is nearly eleven times less than in the group of 294,108 uninoculated persons in these villages.

(c) Other conditions remaining constant, the occurrence of a second or third outbreak of cholera in a village in the course of the epidemic in the district seems to be influenced by the proportion of the population inoculated in the first outbreak.

The study of the subject is being continued. Thanks are due to the Health Officers of the several districts and their subordinate staff for the collection of the data in the present survey.

Annexure I.

Interval.	No. of villages with two outbreaks.	No. of anticipatory inoculations.	No. of attacks among anticipatory inoculations in 2nd outbreak.	Rate per mille.	No. of unprotected.	No. of attacks among unprotected in 2nd outbreak.
1	2	3	4	5	6	7
Villupuram Range (163 villages)						
14 days	63	26,247	22	0.82	34,047	508
21 days	49	21,601	21	0.97	21,550	438
30 days	38	17,821	20	1.12	15,583	370

	Difference in rates.	X^{-2}.	P	
	8	9	19	
(14 days)	14.9	337.2	(Less than)	.0016
(21 days)	20.3	383.9	do.	.0016
(30 days)	23.7	173.9	do.	.0016

Summary of preliminary study on anti-cholera inoculation survey in South Arcot District.

According to Russell's studies on the epidemiology of cholera in Madras Presidency, the two important features are its periodicity at seven yearly intervals and its several correlation to the monsoons. Some districts derive the major portion of their rainfall from the South-West monsoons, some from the North-East monsoon whilst the rest get from both. The first two groups have a single peak whilst the third has two peaks. Migrations of populations especially in connection with fairs and festivals and agricultural operations in connection with transplantation and harvesting are associated factors in originating and spreading cholera epidemics.

2. There was a periodic wave of cholera in 1935-36 when above 70,000 deaths from this disease were recorded. The present epidemic commenced in the second quarter of 1942 and has continued in 1943. Case fatality rate ranged from 50 to 60 per cent ; the total cholera mortality in 1942 was approximately 52,000. About two-thirds of the Province was involved last year.

3. One of the most important preventive measures in cholera epidemics is anti-cholera inoculation. Approximately 4.4 million persons in the affected districts in Madras Presidency were inoculated in 1942.

4. Investigation based on large amount of statistical material do not appear to have been carried out for the assessment of the immunity of anti-cholera vaccine either as a personal prophylactic or herd immunity in the prevention or control of cholera epidemics. As a very large number of inoculations had been done in the 1942 epidemic, the suggestion was made at the last meeting of the Scientific Advisory Board that the experience in Madras should be availed of for the above investigation. The suggestion was accepted by the Board as well as by Madras Government.

5. Even before the investigation was commenced, it was suggested that the best data would be those relating to a community which should be inoculated before the occurrence of the epidemic so that they might be compared with the experience of an uninoculated cholera affected community. For several reasons, the adopton of this suggestion was impracticable. There were however instances of several villages which, in the course of the epidemic, had more than one outbreak of cholera. The inoculations done during and a week before the second outbreak are virtually anticipatory inoculations for the second and the succeeding outbreaks.

6. Vaccine containing Inaba and Ogawa strains manufactured at the King Institute was used in the epidemic. In the larger proportion of cases, the vaccine was given in a single dose—1 c.c. for adults and .5 c.c. for children.

7. Data for the investigation were collected in prescribed cards ; one card was used from every village or suburbs of a village—called hamlets in Madras Presidency—according to the distribution of cases in the whole village or hamlet.

8. The survey has been carried out in close collaboration with Dr. C. G. Pandit and Dr. K. V. Venkatraman, Director and Assistant Director, King Institute of Preventive Medicine, Guindy. The statistical portion of the work has been done by the Statistician in the office of the Director of Public Health, Madras Mr. Parthasarathy. The District Health Officers concerned and their subordinate staff have taken considerable pains in the account collection of the data.

9. For administrative reasons, it has been possible to restrict the present preliminary study to only one district, viz., South Arcot. There were approximaterly 30,000 cases and 16,400 deaths in 1,600 villages. The population exposed to risk was 1.02 millions and about 0.76 million persons were inoculated. The data of 1,283 villages or hamlets have been included in the present report.

10. *Does anti-cholera inoculation confer immunity against cholera ?*

(i) (a) In an aggregate population of 1.33 millions exposed to risk, there were 391,334 ' protected ' persons (according to the definition in the International Quarantine Regulations) and 941,901 unprotected persons including uninoculated and imperfectly protected persons. The number of cases of cholera in these two groups was 571 and 16,198 respectively, the proportional case incidence being 1.5 to 17.1. The incidence in the second group was 11.4 times as in the first group.

(b) It has been stated that, in the case of villages which experienced more than one cholera outbreak in the epidemic, the inoculations done in the first outbreak and in the succeeding interval prior to six days before the date of onset

of the second outbreak, could be considered as anticipatory inoculations with respect to the second outbreak. 387 out of 1,283 affected villages experienced a second outbreak of cholera.

The following is a summary of the cholera incidence in the above protected group of anticipatory inoculations and in uninoculated people. In this classification, the fallacy due to the inclusion of partially immunized persons in (1) above is avoided. In the group of 140,745 protected persons (anticipatory inoculation), there were 149 cases of cholera ; in the group of 294,808 persons, there were 3394 cases. The ratio in the two groups is 1.06 to 11.51; the incidence rate in the latter group was 10.9 times the rate in the former. The corresponding figure in (a) above is 11.4.

The differences in the ratios in (a) and (b) are statistcally significant.

(iii) TIME INTERVAL TAKEN FOR DEVELOPMENT OF IMMUNITY.

Out of 2,637 cases of cholera among the inoculated, about 200 occurred on the day of inoculation and 400 on each of the following three days. The number steadily declines from the fourth day onwards and is at its minimum (30) on the 10th day after inoculation. It is seen from this that immunity manifests itself on the 4th day and the maximum level is reached on the 10th day.

(iii) *Influence of herd immunity.* According to present analysis, in the case of villages where less than 30% of the population had been inoculated in the first outbreak, there were two or more outbreaks of cholera. The total number of villages which had one outbreak was about 900 and more than one, nearly 400.

(iv) *Mortality from cholera in the inoculated population.*

Whilst the protected population is atleast 10 times less susceptible than the unprotected in getting cholera, the case fatality rate, viz., 46.7% though less than in the unprotected (63.5%) is high.

MALARIA.

4. Malaria Institute of India, Research Section, Delhi.

The research activities of the Institute have again been severely curtailed owing to the prevailing abnormal conditions. The post of the Malaria Research Officer has remained unfilled and a large proportion of the work of the staff has been devoted to instructional courses in malariology for officers deputed from the various branches of the Defence Department. Five of these courses have been held during the year at which 124 officers have been trained. Special training in malariology was arranged for two IAMC officers; two Chinese engineers; one medical officer from Ceylon ; one malaria overseer of the Health Department of the Tata Iron and Steel Co. Ltd., Jamshedpur and one malaria supervisor. Facilities were also given to the Central Command, Agra to hold a short malaria course for military medical officers.

Publications.

Journal of the Malaria Institute of India.—Two numbers containing 19 papers and 3 abstracts have been published during the year.

Scientific material sent to other workers.

Strains of monkey malaria and an infected monkey have been supplied to the Director, Pasteur Institute, Shillong. Stained blood films illustrating the various stages of the malaria parasite, preparations of infected mosquito guts and glands, mounted specimens of mosquitoes and larvae, *Gambusia* fish and samples of strains have been supplied to workers in various parts of India.

Testing of insecticides and sprayers.

An extensive series of experiments have been conducted to test the mosquitocidal value of a number of insecticides received from Great Britain and U.S.A. Experiments with so-called activators of pyrethrins (Lethane 384, Lethane Special, IN 930 and sesame oil) have shown that these have little or no effect in increasing the kill of mosquitoes under Indian conditions. Other substances, such as *cardol, neem oil, extract of neem* have also shown no value in the spray-killing of adult mosquitoes. Pymulso, watery emulsion spray (Stafford Allen), containing 0.02 per cent pyrethrins and 2 per cent rotenone, gave a good kill, but was too thick for satisfactory atomization. Pymulso concentrate proved more effective but its immediate knock-down was poor. Concentrates containing DDT (acive principle of Neocid) have given good results as regards final kill but showed practically no immediate knock-down. A standard emulsifier (MII), locally prepared, has proved biologically stable up to 9 months even after dilution with water. The final spray prepared contains 0.025 per cent pyrethrins, i.e. 1/4 the quantify contained in the army oil spray. This spray has been used extensively during the malaria season in Delhi and has proved effective against flies as well as mosquitoes.

The standard type of hand sprayer (MISH) has been modified in certain respects with a view to attaining greater simplicity.

Testing of repellents.

A large number of experiments have been carried out with various types of antimosquito creams. Vanishing creams containing 0.4 per cent pyrethrins have yielded the most lasting results. Extracts of *titapathi* and *kuth*, two indigenous aromatic plants, showed no repellent properties. Dimethyl phthalate

in a vanishing cream with stearic acid base was also ineffective but gave good results in liquid form and in a cream with a tragacanth base.

Testing of larvicides.

A number of chemicals and oils have been tested for their toxicity. Cuprous cyanide proved twice as toxic to anopheline larvae as paris green applied either as a dust or by the dustless method. A mixture of used engine oil 90 parts, kerosene oil 2, diesel oil 5 and Attock antimalarial oil or Malariol 3 has proved to be an effective and economical larvicide.

Entomological research.

The study of mosquito bionomics in respect of the colonies maintained in the laboratory has been continued. A new colony of *Aedes albopictus* has been started. Numerous collections of mosquitoes from workers in different parts of India have been identified.

Field researches.

(a) *Delhi.*—Epidemiological studies have been continued in certain localities of the riverain section and further intensive investigations were carried out during the post-epidemic period affecting Delhi Province. Antimalaria Operations in Delhi have been directed and supervised as in previous years.

(b) *North Kanara.*—The research unit deputed from the Malaria Institute of India conducted malaria investigations in North Kanara over a period of 13 months. Malaria is prevalent in an endemic form throughout the greater part of the district. Hyperendemic conditions exist in the ghat section, while the coastal area is comparatively free. *A. fluviatilis* has been incriminated as the sole vector of any importance. The period of transmission extends from October to June.

Laboratory researches.

(1) Monkey malaria strains have been maintained as heretofore. (2) High titre human and bovine antisera for determining the source of mosquito blood meals by the precipitin test have been prepared from fowls and rabbits. 5,731 mosquito blood meals including 2,830 sent from China through the Rockefeller Foundation, have been tested. (3) A number of stains for use in the diagnosis of malaria parasites have been tested. (4) A water soluble stain for the rapid and economical diagnosis of malaria parasites in both thick and thin smears yielding very satisfactory results under all conditions has been prepared in these laboratories. (5) 2,416 blood smears from fever cases have been examined. (6) Certain indigenous drugs have been tested on monkey malaria, but none had any effect on the course of the disease.

Southern India branch.

Mosquito infectivity experiments with *P. knowlesi* and *P. gallinaceum* were continued. No mosquito infections were obtained with *P. knowlesi.* Six species of *Aedes* were infected with *P. gallinaceum.* A study of the eggs of *A. fluviatilis* has shown that the two different types of egg patterns previously observed are not hereditary in character.

A number of studies on the bionomics of *A. fluviatilis* have also been carried out. A laboratory colony of this species has been successfully established. Colonies of *A.stephensi, P.gallinaceum, Aedes albopictus* and *Armigeres obturbans* have been maintained.

NUTRITION.

5. Nutrition Researches under Dr. W. R. Aykroyd at the I. R. F. A. Nutrition Research Laboratories, Coonoor.

Work carried out by the Nutrition Research Laboratories has included the following :

The analysis of foods.

Study of the composition of foods is an important part of work of the Laboratories. Thirty years ago the investigation of the subject was a comparatively simple matter, because only a few of the constituents of food which are of importance in nutrition had been recognised. To-day a large number of such constituents is known and suitable methods for determining their presence in foods have been developed.

Vitamins of the B group are of great importance in human nutrition. Further work has been done on methods of testing for vitamins of this group. A simple enzymic method, in which an enzyme preparation from pig's intestinal mucosa is used for the extraction of vitamins from test materials, was evolved and used in routine tests for vitamin B and nicotinic acid. It was applied to numerous samples of yeast and yeast extract and also to wheat and rice samples sent by the Food Department. When pure vitamin B_1 was added to yeast extract (marmite substitute) in the proportion of 30 μg/g., there was complete destruction of the added vitamin in 2 months at 37°C. At Coonoor room temperature, ninety per cent loss was observed in 6 months. The fortification of yeast extracts of low vitamins B_1 potency by the addition of pure vitamin B_1 is thus not to be recommended.

In the course of this work it was found that certain raw cereals and cereal products e.g. ragi (*Eleusine coracana*), bajra and rice polishings, contain a thermolabile factor which destroys vitamin B_1 in vitro, and this may be similar to that found by American workers in carp. Pigeons fed on raw ragi did not, however, develop polyneuritis, i.e., vitamin B_1 deficiency. By a coincidence, a pond in Sim's Park adjacent to the Laboratories has recently been stocked with carp as part of the campaign for producing more food, so that a source of the "anti-vitamin B_1" factor described by American workers will be available.

It is not only the higher forms of life that require vitamins. During recent years it has been discovered that many bacteria cannot grow and thrive unless their food contains some of the various vitamins needed by human beings. This fact has been used as the basis of delicate quantitative tests for vitamins. In the Laboratories such methods of testing are now being developed, using the organisms *L-bulgaricus*, *L-acidophilus* and *Streptococus lactis*. Insect larvae also require vitamins and a method for the estimation of vitamin B_6 (pyridoxin) using the larva of the rice moth has been evolved. Previously a chemical method of testing for this vitamin was evolved in the Laboratories by Swaminathan, based on the colour reaction between vitamin B_6, diazotised sulphanilic acid and p-nitranaline. The larva method will serve as a check on results obtained by the chemical method.

The relative nutritive value of animal ghee and vegetable ghee (vanaspati) is a controversial question of considerable importance. Detailed information about the vitamin A content of animal ghee has hitherto not been available so that the advantage of consuming animal instead of vegetable ghee, in respect of vitamin A intake, could not be estimated. To throw light on the problem, the

vitamin A content of 78 samples of animal ghee has been determined. The average value for pure cow's ghee, obtained from well fed cows, was 34 international units per gramme. The average value for pure buffalo ghee— 10 I.U./g.— was considerably lower. Thirty-two samples of ghee as ordinarily purchased and consumed (South Indian samples) had an average potency of 9 I.U./g. The heating of ghee at 130°C. for 20 minutes led to the destruction of 45 per cent of the vitamin A originally present. Ghee used for frying puris was found to lose vitamin A rapidly. Clearly, taking into consideration the amounts of animal ghee usually consumed and losses of vitamin A in cooking, the proportion of vitamin A requirements obtained from this source by consumers in India is small.

Three samples of cow's ghee were tested for vitamin D by the " line " test. The values were considerably higher than those reported for butter in England. Further tests on the unsaponifiable fraction gave only slightly lower values. These results clearly require extension and confirmation.

Dehydrated vegetables are now being manufactured in large quantities in many belligerent countries and it is important that their nutritive value should be studied. A good deal of work on this subject has been carried out in the Laboratories. Steam-blanched cabbage was found to lose vitamin C more rapidly on storage than cabbage blanched by dipping in boiling water. Loss of vitamin C in dehydrated vegetables prepared by the so-called " pre-cooking " method was more rapid than in vegetables prepared by other processes. The general conclusion arising out of a considerable amount of work on the vitamin C content of dehydrated vegetables is that these cannot be relied upon as antiscorbutics after a period of a few months storage. While carotene is somewhat more stable than vitamin C in dehydrated vegetables, very appreciable losses occur on storage. After 20 weeks storage at 98°F, bitter gourd, cauliflower, carrot, pumpkin and potato lost from 35-65 per cent of their original carotene content. Losses in the mineral content of dehydrated vegetables during reconstitution and cooking amounted to 60 per cent. When in U. S. A. attending the United Nations Conference on Food and Agriculture, Dr. Aykroyd collected considerable literature on the effect of dehydration on the nutritive value of vegetables and other foods, a subject which is being intensively studied in England, Canada, U.S.A. and Australia.

Numerous samples of shark liver oil have been tested for vitamin A by colorimetric and spectrophotometric methods. No relation was observed between the stability of vitamin A in shark liver oil and peroxidase values.

Animal experiments.

The relation between leprosy and nutrition is a problem of considerable importance. Rats and other animals cannot be infected by the human lepra bacillus, but a condition which appears to be analagous to human leprosy can be produced in rats by an organism closely resembling the lepra bacillus. In a series of experiments, rats receiving the good Coonoor stock diet were successfully inoculated with rat leprosy. This experiment was carried out at the request of Dr. Lowe, School of Tropical Medicine, Calcutta, to confirm or otherwise a published report that rats on a good diet living in good climatic conditions cannot easily be infected by rat leprosy. Further experiments in the relation between diet and susceptibility of rat leprosy are in progress.

Fluorosis or fluorine poisoning caused by the presence of excessive amounts of fluorine in drinking water is a matter of some public health importance. The

" mottling " of dental enamel is an indication of mild degrees of fluorosis which in severe degrees may cause crippling bony deformities. The protective effect of calcium, salts on fluorine poisoning in rats has previously been reported. Magnesium salts were found to exert a similar though somewhat less marked effect, the amount of magnesium needed for this purpose being, as in the case of calcium, many times that necessary for stoichiometric combination with fluorine. The mechanism by which the protective effect of calcium is exerted has been investigated in a long series of experiments, but so far the problem has not been solved.

Much of the high infant mortality recorded in India is due to malnutrition. It was felt to be of interest to study the effect on rat-suckling-mortality of supplying mother rats with poor rice diet of the kind commonly consumed in India. The mortality on a poor rice diet amounted to 60 per cent, of which some 20 per cent was due to " cannibalism " on the part of the mothers. The addition of milk or yeast to the diet eliminated " cannibalism " and reduced mortality to about 28 per cent.

The pyruvic acid content of rice moth larvae subjected to strenuous " exercise " was found to increase ; this occurred in both normal and vitamin B_1-deficient larvae, the initial level in the latter being much higher. A similar increase has been recorded in the blood of human beings suffering from vitamin B_1-deficiency. The time taken for pyruvic acid values to return to normal was about 3 hours in both normal and deficient larvae. Methyl-glyoxal had no obvious toxic effect on vitamin B_1-deficient larvae. The incentive behind this observation was the claim of Japanese workers, supported by Fehily*, that infantile beriberi is caused by the toxic effect of methyl-glyoxal in the milk of vitamin B_1-deficient mothers.

Rice moth larvae require riboflavin and pyridoxin for growth, but not nicotinic acid and pantothenic acid. All these are vitamins of the B_2 group. The larvae can readily be used for the assay of riboflavin and pyridoxin in foods, the technique involved being relatively simple. A linear relationship exists between larval growth on a pyridoxin-deficient diet and the concentration of added pyridoxin up to about 1 microgram per g. of diet. It may be added that the larval technique offers an almost unlimited field for the investigation of the distribution, interaction and functions generally of the B group of vitamins.

Clinical and field investigations.

In the course of investigations on the treatment of superficial keratitis by riboflavin, it was observed that some cases of superficial keratitis due to riboflavin deficiency were also suffering from angular conjunctivitis, associated with the presence of the Morax-Axenfeld bacillus. On treatment with riboflavin, the keratitis and conjunctivitis tended to clear up simultaneously, while the M.A. bacillus disappeared from smears. Cases of angular conjunctivitis without superficial keratitis were treated with riboflavin with similar results. Cultures of M.A. from cases of angular conjunctivitis were inactivated *in vitro* by riboflavin. Angular conjunctivitis associated with the presence of M.A. is a common condition ; it is usually successfully treated by zinc ointment but tends to recur after treatment. Convincing evidence that riboflavin deficiency plays a part in its etiology would be of clinical importance. Unfortunately this promising investigation was interrupted at an incomplete stage by the departure of the worker concerned to take a commission in the I. A. M. C. It is hoped to continue it when circumstances permit.

* *The Caduceus* (University of Hong Kong), 1940, 19, p.78.

A survey of gingivitis and periodontal disease was carried out in Lahore, with the collaboration of Dr. C. D. Marshall Day, Superintendent of the de Montmorency College of Dentistry. These conditions are found in varying degrees, ranging from slight thickening of the gums in earlier and milder stages to a later stage of marked hypertrophy with marginal ulceration and the formation of pus-pockets (pyorrhoea). Among males of middle age the incidence of pyorrhoea was found to be almost 100 per cent, while, contrary to experience in Western countries, gingivitis is common among children and is sometimes observed in children as young as 5 years of age. An experiment was carried out to discover the effect of vitamin C on gingivitis and associated conditions in children in an orphanage. One hundred mgs. of vitamin C were given to 50 children for 100 days, a control group receiving no supplement. No change in the gingival conditions was observed as the result of giving vitamin C. No obvious change occurred in the general condition of the children, although the diet of the institution appeared to be low in vitamin C.

The relation between " mottled enamel " and dental caries was studied in various parts of the Punjab, the fluorine content of drinking water being determined as part of the investigation. Within a certain range (2-6 parts per million) no clear-cut relation was observed between the fluorine content of water and the incidence of mottled enamel. When the content reaches 8-10 ppm., mottling is severe. A slightly lower prevalence of caries was observed in groups showing higher degrees of mottling.

Some 70 cases of infantile beriberi were successfully treated in Coconada by the injection of vitamin B_1. An attempt is being made to study the incidence of infantile beriberi by following up the history of most infants born in the municipality for 6 months.

An investigation of " nutritional diarrhoea " has been started in Madras. A series of cases has been observed and described. Preliminary results suggest that injection of nicotinic acid is of considerable value in the treatment of this condition.

Training course.

Arrangements were made to give the usual course in April-June. Owing, however, to the departure of the Director early in April to attend the United Nations Conference on Food and Agriculture as a delegate of the Government of India, the course was cancelled. The Director was absent from the Laboratories until the last week in July.

Educational and advisory work.

Enquiries about various aspects of nutrition were received from all over India. Advisory work, official and non-official, has been considerably increased as a result of the food situation.

6. Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

Bio-synthesis of ascorbic acid under the influence of narcotics.

In continuation of the researches on the increased urinary excretion of ascorbic acid by rats when fed with a variety of organic substances, including nerve depressants, some work has been done in an attempt to throw some light

on the process of ascorbic acid synthesis under certain physiological conditions, analogous to those produced by nerve depressants, namely (a) lowering of oxygen consumption (b) disturbance in the normal carbohydrate metabolism and (c) inhibition of the activity of respiratory enzyme systems. Under these physiological conditions, however, no increased elimination of ascorbic acid was observed in the urine of rats, as is observed under the influence of narcotics.

(1) Increased ascorbic acid synthesis under the influence of narcotics may be due to some disturbances in the carbohydrate metabolism. In order to explore the nature of these possible disturbances sugar and inorganic phosphorus in the urine and blood and the glycogen content of liver of normal and narcotised rats have been studied.

Sugar in 24 hours' urine of normal and narcotised rats has been determined by the method of Shaffer and Somogyi as modified by West (*Bioch. J.*, 1932, **26,** 1720). The results so far obtained do not show a signfiicant difference between the two sets of animals. Blood sugar has also been determined by the method of Shaffer and Somogyi (*J. Biol. Chem.*, 1933, **100**, 695). Some tentative results have been obtained, which seem to indicate some significant difference in the blood sugar of normal and narcotised rats.

Inorganic phosphorus in the urine and blood of normal and narcotised rats has been determined by the method of Fiske and Subbarow (*J. Biol. Chem.*, 1925, **66**, 375). So far as urinary phosphorus is concerned, there seems to be no difference in the values of the two sets of animals, but blood phosphorus of narcotised animals seems higher than that of normal.

Liver-glycogen of normal and narcotised rats has been determined by the method of Pflüger as modified by Good *et al* (*J Biol. Chem.*, 1933, **100**, 485). From the results so far obtained it seems there is some depletion of liver-glycogen of narcotised rats as compared to normals.

(2) Increased synthesis of ascorbic acid by narcotised rats may also be derived from the glucogenic residues of amino acids, which would mean increased protein metabolism in narcotised animals. To test this, experiments were carried out for determining the nitrogen values of 24 hours' urine of both normal and narcotised rats. The results so far obtained have not indicated any significant difference between the nitrogen values of the urine of normal and narcotized rats.

(3) To study the effects of injection of certain products which are normally derived from the breakdown of carbohydrates, experiments were carried out on narcotised rats injected with pyruvate, lactate and glucose and the ascorbic acid content of urine, liver, kidney and brain tissues was determined, which showed some increase over that of the normal controls.

7. Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

Analyses of fish.

The results of analyses of the edible portions of 54 varieties of Bengal fresh-water fish regarding their water, protein, fat, calcium, phosphorus, total and ionisable iron content, were reported earlier (Saha & Guha, *Ind. Jour. Med. Res.* 1938, **26**, 921 ; ibid, 1939, **27**, 873 ; Saha & Ghosh, *Ann. Biochem. Expt. Med.*, 1941, **1**, 159). In continuation of the above work 5 more varieties of fish

have been investigated. From the results obtained it has been found that among the new varieties of fish analysed, *Shilong* has the highest fat content, 9.3%. A very big and uncommon variety of *Magur* (*Clarius batrachus*) containing 21.6% protein has been found to be the richest in protein. As regards the calcium content *Chital* (*Notopterus chitala*—smaller variety) has the highest value, 0.63%. The fish *Gazar* has an unusually high phosphorus content, 0.92%. This fish is also a rich source of available iron.

In continuation of the work reported earlier concerning the nicotinic acid content of different varieties Bengal fresh-water fish 13 more varieties of fish have been studied. From the results it has been found that *Bhangar* (*Mugil tade*) has the highest nicotinic acid content, viz. 1. 77 mg. per 100 g. of fresh muscle tissue and it has been found to be the richest in nicotinic acid among all the varieties of fish hitherto investigated. *Folui* (*Notopterus notopterus*) is the next richest source of nicotinic acid, containing 0.89 mg. per 100 g. of tissue. The poorest source of nicotinic acid is *Punti*, which gives a figure of 0.18 mg.

A full table giving all the results of analyses so far done is now being prepared. The appended table gives a summarised list of those varieties of fish which have so far been found to be rich sources of one or other of the nutritive constituents.

Effect of cooking on the biological value of fish protein.

Results concerning the biological values of the proteins of a few species of fish after different processes of cooking, investigated by the rat growth method, have been reported earlier. This has indicated that except in the case of *Hilsa* fish (*Clupea ilisa*) whose biological value of the protein was found to be the greatest with fried fish and least with raw fish the best results regarding the biological value of these fish proteins were obtained with underdone fish as compared with raw, fully cooked or fried fish. The changes in the biological values of 4 more common varieties of fish proteins *viz.* those of *Parsey* (*Mugil parsia*), *Boal* (*Wallago attu*) *Sarputi* (*Barbus sarana*) and *Bhangar* after different processes of cooking have been studied. These findings are in agreement with the results reported earlier, which would indicate that so far as the protein value is concerned underdone fish is, in general, superior to raw, fully boiled or fried fish.

Available iron in fish.

It has been reported earlier that about 30 to 40% of the total available iron in fish tissue is present in complex combination with nucleoprotein and copper. Studies on the distribution of non-protein nitrogen and isolation of individual purine and pyrimidine bases indicate that the nucleoprotein contains adenine, guanine, uracil and cytosine. The sugar present in the compelx has been found to be *d*-ribose which would indicate the resemblance of the nucleic acid of this complex with yeast nucleic acid. The interest in this complex lies in the fact that it appears to stimulate haemopoiesis in experimental animals.

SUMMARISED LIST OF THOSE VARIETIES OF FISH WHICH HAVE SO FAR BEEN FOUND TO BE RICH SOURCES OF ONE OR OTHER OF THE NUTRITIVE CONSTITUENTS.

Figures are given per 100 *g. of fresh raw fish.*

Bengali name.	Zoological name.		Bengali name.	Zoological name.	
	PROTEIN.			CALCIUM.	
Mahasole	Barbus (Tor) tor	25.2 g.	Parsey (small)	Mugil parsia	1.05 g.
Singhi	Saccobranchus fossilis	22.8 „	Bata (small)	Labeo bata	0.79 „
Hilsa	Chupea ilisa	21.8 „	Singhi	Saccobranchus fossilis	0.67 „
Magur (big & uncommon)	Clarius batrachus	21.6 „	Parsey (big)	Mugil parsia	0.65 „
Folui	Notopterus notopterus	19.8 „	Chital (small)	Notopterus chitala	0.63 „
	FAT.			PHOSPHORUS.	
Hilsa	Clupea ilisa	19.4 g.	Gazar Parsey (small)	 Mugil parsia	0.92 g. 0.70 g.
Dhain	Silonia silundia	12.1 „	Singhi	Saccobranchus fossilis	0.65 „
Pangas	Pangasius pangasius	10.8 „	Bhole	Sciacna coitor	0.58 „
Sarputi	Barbus sarana	9.5 „	Lata	Ophicephalus punctatus	0.53 „
Shilong		9.3 „			

Figures are given per 100 *g. of fresh raw fish.*

Bengali name.	Zoological name.		Bengali name.	Zoological name.	
	TOTAL IRON.			IONISABLE IRON.	
Mahasole	Barbus (Tor) tor	3.83 mg.	Bata (small)	Labeo bata	1.01 mg.
Parsey (big)	Mugil parsia	3.23 „	Singhi	Saccobranchus fossilis	0.97 „
Chital	Notopterus chitala	2.98 „	Chela	Chela bacaila	0.96 „
Singhi	Saccobranchus fossilis	2.26 „	Palmplate	Pampus argenteus	0.83 „
Hilsa	Clupea ilisa	2.13 „	Mahasole	Barbus (Tor) tor	0.80 „
	COPPER.			NICOTINIC ACID.	
Chela	Chela bacaila	0.510 mg.	Bhangar	Mugil tade	1.77 mg.
Lady vendi	Sillagpo sihasna	0.230 „	Magur	Clarius batrachus	1.02 „
Bata (small)	Labeo bata	0.170 „	Lata	Ophicephalus punctatus	1.00 „
Chital	Notopterus chital	0.165 „	Boal	Wallago attu	0.98 „
Koi	Anabus testudineus	0.162 „	Dhain	Silonia silundia	0.91 „

8. Studies in human nutrition with Indian diets under Dr. K. P. Basu at Dacca University, Dacca.

METABOLISM OF FATS.

(1) *Percentage of absorption of different fats & oils.*—Investigations on the percentage absorption of different vegetable oils and fats and also of fats from animal origin, that had been started in the previous year, have been repeated with other experimental subjects. It has been definitely proved that all the oils and fats show high absorpton, the percentage of absorption varying between 92 and 100. The fats and oils used in the study were mustard, cocoanut, sesame

and groundnut oils, margarine, butter fat (both from cow and buffalo) and vanaspati and the percentages of absorption were 92, 99, 95, 100, 100, 98, 95.5 and 100 respectively.

(2) *Rate of absorption of various fats.*—Rates of absorption of the different oils and fats after definite time intervals are being determined by experiments on rats. For this purpose 1 c.c. of the melted fat is fed to a rat by means of a stomach tube and the portion of it remaining in the stomach and intestine after a definite interval is found out. The fats and oils that have so far been examined are butter fat, cocoanut oil and olive oil. It has been found that 42 p.c., 48 p.c. and 59 p.c. of (cow) butter fat is absorbed at 2 hrs., 3 hrs. and 4 hrs. interval. Cocoanut oil has a slower rate of absorption. After 2 hrs. 36 p.c. is absorbed while after 3 and 4 hrs. the percentage absorbed is 43 and 49 respectively. Olive oil appears to have a slightly higher rate of absorption than butter fat. It is absorbed to the extent of 41, 49 and 61 p.c. at 2 hrs., 3 hrs. and 4 hrs. interval respectively. Further experiments are in progress using other fats and oils and more rats.

(3) *Influence of different fats on calcium and phosphorus metabolism.*—Experiments have been started to determine the influence of the nature of the fat ingested on calcium and phosphorus metabolism and very interesting results have been obtained in this line with human subjects. All the experiments go to prove that the presence of fat in the diet increases the utilization of calcium and phosphorus and in general fats appear to exert a definite calcifying action which is however, not the same with all types of oils and fats. It has been found that amongst the fats and oils studied, butter fat has the most beneficial effect on the absorption of calcium. The beneficial effect of oils like sesame, groundnut, mustard and buffalo-butter fat was also appreciable. On the other hand the cocoanut oil causes a large part of the calcium to be eliminated through the faeces and exerts an adverse effect on the retention of calcium. In view of the importance of this finding further experiments are in progress to determine the actual mechanism by which fat influences the utilizaton of calcium and phosphorus.

(4) *Effect of aneurin on the transformation of carbohydrate into fat.*—No definite conclusion has been arrived at from the experiment which was started to determine whether aneurin stimulates the formation of fat from carbohydrates. In this investigation three series of rats, all of which were maintained at a low fat diet, were used. The first series consisted of rats which received the components of the vitamin B-complex in a purified form (aneurin, nicotinic acid and riboflavin). The second series received exactly the same diet as the first but aneurin was excluded from the diet. The third group received the diet supplemented with yeast extract. After a period of 5 weeks all the rats were killed and their body fats were determined by standard methods. No significant difference in fat content of the body of the rats was observed in the different series. The experiments will be repeated.

(5) *The stability of vitamins in different fats*—The stability of vitamin A added to different vegetable oils is being determined by the antimony trichloride method with the help of the Lovibond Tintometer. The experiments are in progress.

Metabolism of lead.

The metabolic data for lead reported last year were abnormally high. This has been accounted for by the unstability of the standard lead chromate

solution in HCl acid used in the analytical micro-method for the estimation of lead (Letonoff & Reinhold, *Ind. Eng. Chem.*, Anl. Ed. 12, p. 280, 1940). Contrary to the claims of Letonoff & Reinhold this solution has been definitely proved to deteriorate rather quickly. This deterioration of lead chromate may be averted by taking at the time of each determination a standard lead acetate solution and precipitating lead potassium chromate by the addition of potassium chromate.

The different reagents and chemicals were purified and freed from lead by repeated crystallisation and this modified technique was followed with excellent results. The intake of lead on a vegetarian rice diet was found to be 0.412 mg., the urinary and faecal excretions being 0.0015 mg. and 0.213 mg. respectively. Two varieties of ' Rice fish diet ' with 600 gms. and 400 gms. of rice gave an intake of 1.24 mgs. and 3 mg. of lead respectively and the faecal lead, in the latter case was found to be 0.076 mg. Another experiment on a whole-wheat diet was performed and the intake of lead was 1.666 mgs.

Further experiments on human subjects with other varieties of diet are in progress.

9. Studies in the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

During the current financial year experiments on the metabolism of carotene in animal organs have been carried on. A number of rats of about 40 gms. of weight on the average were kept on a basal diet free from vitamin A and carotene. After a month or so when they began to lose weight and xerophthalmia was found to have developed a few of them were killed and their liver, spleen and kidney were examined to detect any store of vitamin A in them. No store of vitamin A was discovered in those organs. Thereafter, the remaining animals were divided into several groups which were given different doses of pure β—carotene for different periods of time. Ten gm. of β—carotene were given per day to each of the animals of the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th, groups for 3, 6, 9, 12, 18, 24 and 30 days respectively. When the experimental periods were over the animals were killed and their liver, spleen and kidney were examined for vitamin A stores by the improved spectrophotometric method recently developed. It was however, noticed that there was no store of Vitamin A in those organs examined.

The experiments were then repeated by increasing the dose of carotene and the animals of the groups 8th, 9th and 10th., were given β—carotene per rat per day at the level of 20γ for 3 days, 20γ for 6 days and 40γ for 6 days respectively. Even after such high doses of carotene feeding there was no excess of vitamin A stored up in liver and other organs. Almost half of the carotene administered orally was excreted along with the faeces although there was cure of xerophthalmia and definite increase in body weight.

Next time the dose was increased to 60γ of β—carotene per rat per day and it was administered to the animals of the 11th group for 16 days. On examination of the organs it was however found that vitamin A was stored up only in the liver and neither in spleen nor in kidney. The major portion of carotene was excreted along with the faeces and only a small amount as much as 75 I.U. of vitamin A was in excess of functional needs and stored up in the liver.

Experiments are in progress where vegetables rich in carotene are being administered to rats to ascertain whether carotene is better utilised in purified form or from natural sources. Experiments are also going on to determine the factors helping in the assimilation of carotene.

The work on the effect of cooking of puri, fish, meat, vegetables and sweets on vitamin A content of ghee has been completed. It is found that the major portion of the active principle is destroyed when the ghee is heated at about 150° C for 10 to 15 min., but when it is heated at 200° C or above for more than 10 min. practically the whole of vitamin A is destroyed. The results are described in a paper which is ready for publication.

10. Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

Determinations of plasma proteins in normal subjects have been carried out by several workers. These values are not adequate since most of the studies have been made upon a small group of persons whose state of health in many cases was open to question. Besides, the existing studies have been based upon different methods of analysis which do not give comparable results. The many and varied functions attributed to the different protein fractions of the blood have made it increasingly important that a quantitative study should be undertaken in order to set a standard for albumin, globulin including those of euglobulin and pseudoglobulin and fibrinogen present in the blood of healthy individuals. Such a study furnishes the necessary foundations for the interpretation of results in pathological conditions.

Determination of the plasma proteins has been carried out in 80 healthy subjects by means of salting out separation of different protein by Howe's (1921) method and followed by kjeldahl analysis. The subjects for the study were mostly medical students of both sexes who were examined and questioned by a physician when the blood was taken. Every attempt was made to rule out those cases who might present clinical or subclinical variation in any of the protein fractions as a result of recent immunization, malnutrition or due to any chronic disease. Determination of plasma protein in six children who attended the hospital for slight injury has also been included in this study.

The plasma was separated usually within half an hour from the time the blood was collected. Sedimentation rate of the oxalated blood was determined in every case. The present series includes the results of the highest number of plasma protein determinations in adults recorded so far and the results appear to be more consistent.

Albumin.—The albumin fraction shows no significant variation between two sexes, nor with the height or the weight of the subjects. The albumin level based on 30 determinations on males is 4.9 grams with a range of 4.67–5.3 grams per cent. The mean of 40 determinations in females is 4.96 grams with a range of 4.53-5.16 grams per cent. These values are higher than those observed by other workers. The mean value reported by other workers varies widely with respect to each other and in comparsion with the mean observed by us.

Globulin.—The globulin analysis reported in the literature covers the results of 51 determinations of blood of adults, which show a mean value of

2.60 and a range from 1.32-3.79 grams per cent. This mean, although is in good agreement with the mean of 2.66 gram per cent observed by us, yet the results of the present series are more consistent with a range 2.2–2.9 grams per cent. In only 10 out of 70 cases, the globulin values were above 2.7 per cent. In view of this finding any value above 2.8 per cent may be looked for some abnormal process.

Globulin content of blood plasma below the average normal has been observed in persons apparently healthy but underweight. The albulin is also low in these persons with the result that the total protein is also below normal. The effect of improved diet on the protein content of the blood of these subjects has been the subject of further investigation.

Fibrinogen.—The fibrinogen values were found to vary between 0.12 to 0.38 gram per cent. In 60 per cent of the cases the fibrinogen value was below 0.2 per cent. This is in agreement with the value given by Ham and Curtis (1938) in their review on the blood fibrinogen in normal persons. In normal pregnancies, the fibrinogen content of the blood was found to be higher than the normal. In 25 cases of normal pregnancies fibrinogen content was found to lie between 0.38 to 0.64 per cent. An abnormal blood fibrinogen is an evidence for an organic disease process which may be present at the time of observation. In most of the cases of tuberculosis studied by us the fibrinogen content of the blood was much above the normal and with a range from 0.75 to 1.27 gram per cent., indicating possibly the extent of injury to the tissue.

Euglobulin and pseudoglobulin.

The determination of euglobulin and pseudoglobulin has been included in the present study with a view to find out, what part of globulin is increased and whether there is evidence suggesting that the increase in any of the globulin fractions is specific for any disease. The value of euglobulin in normal persons has been found to be higher than those observed by Loyd and Pal by refractrometric method but is in agreement with the value obtained by Mudaliar and Coworkers (1934) as shown in *Table* 1.

Albumin-globulin ratio.

In 1912 Epstein reported 12 patients of nephrosis in which there was a lowering of blood albumin, the globulin remained constant. He expressed his findings as A/G ratio, and since then this expression had become deeply rooted in medical literature. The A/G ratio without reference to the total protein is of little significance. The absolute value of albumin and globulin must be used for the sake of clarity. A fall in bood albumin is more significant in indicating the severity of diseases like tuberculosis or cirrhosis of the liver than an increase of blood globulin.

TABLE I.

Plasma protein value in normal subjects (adult), as reported in the literature.

Author	Number of cases	Abumin	Globulin	Eu	Pseudo
		gram. per cent		globulin	
		Range			
1. Linder and Coworkers (1924)	7	3.62–4.9	2.45–2.89	..	..
2. Salversen (1926)	16 male	3.95–5.24	1.96–3.16	..	..
3. Wiener and Wiener (1930)	20	4.20–5.0	1.52–1.90	..	..
4. Van Slyke snd Moore (1930)	9	4.0–4.5	2.8	..	..
5. Mudaliar and Coworkers (1934) in Madras	9	3.5–5.0	1.15–2.65	*0.6*	1.49
6. Loyd and Pal in Calcutta	10	4.59	2.8	*0.16*	1.79
7. *Present series* (Datta) in Bombay (1942-43)	30 males 40 females	4.67–5.3	2.2–2.9	*0.50*	1.

PLASMA PROTEIN IN DISEASES OF THE LIVER.

Determination of plasma proteins in liver diseases has been undertaken in view of the suggested importance of the liver in the formation of the plasma proteins.

Hypoalbuminemia is a constant alteration of plasma proteins observed at one time or the other in all patients who had chronic or advanced diseases of the liver. Albumin value varying from 1.43 to 3.81 gram per cent was found in 30 cases of liver diseases. In cirrhosis of liver one finds a reduction in albumin and an increase of globulin fraction of the blood which may rise far above the normal. Globulin value varying from 4.06 to 5.37 grams per cent was observed in 6 cases of cirrhosis of the liver. Hyperglobulinemia was associated with hyperproteinemia. We find no reason for the occurrence of hyperglobulinemia and hyperproteinemia in some cases and not in others.

Repeated determination of plasma proteins in the same patients showed that there is direct correlation between the level of albumin and the clinical course. In instances of clinical failure, there was no rise of serum albumin. The total protein and the increase of globulin is of little prognostic value.

Increased globulin in cirrhosis of the liver is always associated with an increase in the euglobulin fraction more than that of pseudoglobulin. Kala-azar is an example of parasitic infection with hepatic involvement which shows highest increase in the euglobulin fraction.

One of the subjects, a doctor, whose blood was examined at an earlier stage and who was included amongst the normals, developed obstructive jaundice. The globulin increased from the normal value of 2.71 to 3.89 per cent, with a corresponding increase in the euglobulin fraction from 0.5 per cent to 1.52 per cent. The sedimentation rate increased from 3mm per hour to 32 mm per hour. With recovery all the values returned to the normal level. The exact significance of the increase in the euglobulin fraction of the blood plasma is not properly understood.

Hyperproteinemia.

Hyperproteinemia is frequently found in most of the chronic and acute infections. Total protein above 8.0 per cent has been observed in a number of cases of tuberculosis, syphilis and also in cirrhosis of the liver. Hyperproteinemia is due to hyperglobulinemia. Increased globulin is again associated with either increase of euglobulin or pseudoglobulin. A study of the relative increase in these two globulin fractions in relation to blood albumin may prove to be of certain clinical significance.

Sedimentation rate in relation to different protein fractions.

Determination of the sedimentation rate has been carried out in all the cases studied by us. There appears to be no quantitative correlation between the sedimentation rate and any of the protein fractions of the blood plasma.

Animal experiment.

Animal experiments both with dogs and rats are in progress. It is intended to study the effect of a diet deficient in protein on the plasma protein and also on the protein content of the different tissues in order to see, if there is any quantitative relationship between the decrease of albumin and the extent of tissue protein undergoing destruction. This study also includes the effect of different food proteins on the regeneration of plasma protein in protein depleted dogs.

11. Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

Experiments with rats.

Animal experiments were carried out to test the effect of varying the levels of fat intake on the growth rate. Young albine rats, 12 in each group, were fed on diets containing 5, 12, 19, and 25 per cent butter fat, one group of 12 rats being given the basal diet (0.85 p.c. fat). The rats were weighed once a week for eight weeks, their average growth per week and daily food intake were noted. During the period of experiments, each rat received a daily supplement of 0.75 gm. of dried yeast, and once a week one drop of diluted Radiostol and shark liver oil which contained 100 I.U. vitamin A and D each. At the end of eight weeks the general condition of rats in all groups was good. The animals were lively, well grown and possessed smooth fur. A few rats from each group were kept for observation regarding their breeding qualities. Rats receiving 5, 12, 19 and 25 p.c. fat produced litters in 3 to 4 weeks. The control rats did not produce litters even after 5 weeks. The determination of serum lipoids in pooled blood serum from each group showed that the iodine value of the total fatty acids—increased with the increase in the intake of the butter fat.

There was no marked difference between the growth rate of rats on different levels of butter fat intake. This might have been due to (1) the different levels of protein in the diet varying between 15 and 11.2 p.c and (2) the low food intake of rats receiving high fat percentage which further lowered the protein consumption.

A second set of experiments was therefore carried out. In this all the experimental diets contained 15 p.c. casein as protein and carbohydrate was

replaced by different quantities of butter fat so that diets containing 5, 12, 19 and 25 p.c. fat were obtained. Groups of six young rats were kept for each level of fat intake, the other conditions being similar to those in the earlier experiment.

The growth rate observed over a period of eight weeks was found to be higher in the groups receiving 5 p.c. and 12 p.c. butter fat in diet, the total gain in weight falling off on either side of this level. The fall in the food intake with increasing fat level and impairment in the reproductive capacity of rats in control group have again been observed. The results of the above experiments are included in the following table (I-A and I-B).

TABLE (I-A)

	Fat per cent in the diet.	Protein per cent in the diet.	Gain in weight per rat per week gram.	Average food intake per rat per day gram	Average intake per rat per day		Average calorific intake per rat per day.
					Fat. gram	Protein. gram	
Experiment I.	0.85	15	10.3	9.1	0.08	1.36	34
	5	14.3	11.8	9.3	0.47	1.33	37
	12	13.2	11.5	8.2	0.98	1.08	34
	19	12.2	10.0	7.0	1.33	0.85	33
	25	11.3	12.5	5.77	1.43	0.64	30
			(I-B)				
Experiment II.	0.8	15	9.8	9.2	0.07	1.37	35
	5	15	11.9	9.3	0.47	1.38	38
	12	15	12.3	8.5	1.02	1.27	37
	19	15	11.0	6.6	1.25	0.99	31
	25	15	11.0	6.2	1.55	0.93	31

A third set of experiments with equalised protein and salt intake has been commenced.

ANALYSIS OF SERUM LIPOIDS IN HUMAN BLOOD.

As has already been reported a number of analysis have been completed. The number of subjects falling in different groups arranged according to their fat intake is not sufficiently large. A preliminary analysis of the results shows, however, that the total lipoids and total fatty acids seem to be unaffected by the amount of fat in the diet but that there is a variation in the iodine value of the fatty acids depending upon the fat content of the diet. Further data are being collected.

The increase in the unsaturation of fatty acids on high fat intake has been previously observed by other workers, and can be confirmed by animal experiments.

The conditions in the animal and human beings are not similar, especially with regard to vitamin intake. The experimental animals on low fat diets receive supplements of fat soluble vitamins A and D whereas in human beings

this is not so. But this difference points to one interesting conclusion that the lowering of unsaturation of fatty acids on low fat intake whch has been observed in both cases is probably independent of vitamins A & D. It is also worth-while to investigate the influence of different dietary fats on the iodine value of the fatty acids of the blood. An experiment is being designed to test this particular aspect.

12. Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

Young growing rabbits 120 to 165 gram in weight were kept on the basal non-manganese diet, outlined in the report for 1942, for a longer period (3 weeks). One group was supplemented with manganese and vitamin B complex, a second group supplemented with B complex only and the control group receiving no supplement. After 3 weeks, the ascorbic acid content of the jejunum, liver and adrenals was determined as shown in Table I.

TABLE I.

Tissue.	Ascorbic acid content (mg per gram)			Ascorbic Acid Synthesis (mg per gram)	
	Mn+B Gr. (I)	B. Gr. (II)	Control Gr. (III)	Gr. (I.)	Gr. (II)
Jejunum	0.30	0.15	0.14	0.16	0.01
Liver	0.12	0.06	0.06	0.06	nil
Adrenals	1.50	0.92	0.90	0.60	0.02

It is evident that deprivation of manganese for a long period makes the tissues of the rabbits poor in ascorbic acid content. The ascorbic acid content of the liver of non-manganese supplemented group is of the same order as that reported for guinea pigs kept on scorbutic diet (Svirbely, *Biochem.* J., 1933).

Fledgings of pigeons were kept in two groups and given a basal diet, similar to that given to the rabbits and as much as possible free from manganese. In order that the young birds may be easily fed the diet was cooked and when of a semi solid consistency cooled and made into small pellets and dried. One group was supplemented with manganese. Both groups received a 50% alcoholic extract of yeast as a supplement of B complex. The pigeons were killed after 12 days on these diets and the ascorbic acid contents of the jejunum and liver were estimated. The results indicate that the supplement of manganese has the effect of keeping the ascorbic acid contents of the tissues normal (.36 mg per gram and .24 mg per gram respectively) whereas in the non-supplemented group the ascorbic acid contents are considerably diminished (.19 mg per gram and .095 mg per gram respectively), the ascorbic acid content of the liver of the non-supplemented group indicating an approaching scorbutic condition. The number of birds employed (3 in each group) did not allow of a statistical analysis.

Young rabbits were divided into two groups (3 each) and kept on the basal non-manganese diet. One group was supplemented with manganese. The 24-hours urine was collected over dilute sulphuric acid (acetic and metaphosphoric acids proved unsuccessful) and the total ascorbic acid excretion for 24 hours estimated. The manganese supplemented group excreted more ascorbic acid (0.55 mg. per animal) than the unsupplemented group (.40 mg. per animal),

There is no doubt that the excretion in the unsupplemented group is also high. This was found to be due to the basal diet containing appreciable amounts of manganese (.39 mg per 100 g). The next experiments were conducted with young white rats (6 in each group). With the same basal diet as given to the rabbits, the supplemented group showed no increased excretion and sometimes showed a smaller excretion. This was thought to be due to toxic effects of manganese in the supplemented group and possible destruction of ascorbic acid in the urine due to increased excretion of manganese. The basal diet was changed and another prepared with ingredients of purer quality. The effect was immediate. The manganese supplemented group excreted more ascorbic acid (.43 mg per animal) than the unsupplemented group (.30 mg per animal). The excretion of the unsupplemented group is lower than that reported by others for normal rats. While the excretion of the supplemented group is of an order which compares well with that reported by others (cf. Musulin *et al*, 1939, J. Biol. Chem., 129, 437). The experiment has lasted long enough (3 months) to admit of any doubt on the specific role of manganese in the synthesis of ascorbic acid by rats. The unsupplemented group also ceased to grow normally. Continuation of the basal diet with the non-supplemented group resulted ultimately in the death of three out of the six animals. One morning two of the rats were found dead. Examination of the rats showed haemorrhages at the knee-joints. To elucidate the point further the animals were subjected to postmortem examination. There were haemorrhages in the lungs and the liver and the intestinal capillaries were severely effected pointing to a condition simulating scurvy. On the next morning the third rat was found dead. No injury marks were visible suggesting murderous action taken by the survivors. But a very interesting incident was that the surviving rats had eaten up the viscera of the dead one. This cannibalistic exhibition by the surviving rats was evidently the outcome of their manganese hunger and the desire for its satiation. The experiment was abandoned at this stage.

It has been possible to explain the increased ascorbic acid synthesis and excretion by rats on the ingestion of some groups of compounds first observed in King's Laboratory and correlate it with the present findings. Rabbits kept on a basal manganese free diet and supplemented with 50 mg carvone, daily synthesise and excrete a greater amount of ascorbic acid. The ascorbic acid content of the jejunum of the carvone supplemented group is much higher than that of the control group (.96 mg per gram and .51 mg per gram respectively) whereas the ascorbic acid contents of the liver of the two groups (.29 mg per gram and .30 mg per gram respectively) are practically the same. The manganese content of the liver of the carvone supplemented group is considerably depleted as compared with the unsupplemented groups which remains normal. This suggests that the higher synthesis in the jejunum in the group supplemented with carvone is due to the mobilisation of the manganese reserve of the liver. There may be a further possible action of these substances in regulating the pH in the intestines.

The ascorbic acid contents have always been determined by the technique developed in the writer's laboratory and gives consistent and reliable results as proved by the recovery of added ascorbic acid and comparison with biological tests. The full details were read out before the Proceedings of the 30th Indian Science Congress and are being communicated to the Press. The writer has no faith in ascorbic acid oxidase treatment in the determination of ascorbic acid and considers it unsound in principle and unwarranted in practice.

13. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. K. P. Basu at the Dacca University, Dacca.

Protein content of soya bean and other pulses.

The protein contents of soya bean samples, received from Lahore are 41.6% (Kashmir variety) and 41.2% (Lyallpur). The sample supplied last year contained 40% proteins.

The protein contents of green gram, dal arhar and Bengal gram, received from Coonoor are 23.4%, 23.6% and 23.5% respectively. The pulses received last year contained 23.3%, 23.6% and 23.8% proteins respectively.

A fresh sample of soya bean has just been received from Lahore and work is going on with it.

Biological value by balance-sheet method.

Experiments on the biological value of the proteins by the balance-sheet method with last year's samples of soya bean and other pulses were repeated on a set of eight rats. The average values for the proteins of soya bean, green gram, dal arhar and Bengal gram have been found to be 47.7, 50.2, 52.1 and 62.8 respectively. The digestibilities of proteins have been found to be 80.5, 79.2, 77.8 and 82.2 respectively.

With fresh pulse samples received from Coonoor and soya bean samples from Lahore, experiments were repeated on six adult rats at 10 p.c. level of protein intake. The average biological values for the proteins of Kashmir soya bean, Lyallapur soya bean, green gram, dal arhar and Bengal gram are 44.1, 48.1, 49.8, 53.1 and 63 respectively.

Biological value by growth test.

Biological values by the growth method have been determined by experiments conducted on 12 young rats for 10 weeks for each pulse and soya bean and the

$$\text{ratio } \frac{\text{gain in body wt. (gm)}}{\text{protein intake (gm)}} \text{ has been}$$

found to be .78, .87, .82 and 1.05 for proteins of soya bean, green gram, dal arhar and Bengal gram respectively. These experiments were carried out with the sample received last year.

Effect of supplementing poor rice diet.

Experiments on the supplementing effect of various pulses and soya bean (Kashmir variety) on the poor rice diet are being continued. As supplements to the poor rice diet (3 parts by weight of soya bean to 22.5 parts of rice diet) the pulses and soya bean seem to have almost the same effect. In preparing the poor rice diet leaves of pumpkin (*cucurbita pepo*) were used instead of cabbage as the latter was not available at Dacca during the summer.

The mean weekly increase in body weight of the rats were as follows :—

	gm.
Rice diet alone	2.62
„ „ & Soya bean (Kashmir variety) ..	3.59
„ „ & Dal arhar	2.83
„ „ & Green gram	3.25
„ „ & Bengal gram	3.50

BIOLOGICAL VALUE BY METABOLISM EXPERIMENTS ON HUMAN BEINGS.

The results of metabolism experiments on a healthy human adult (S.C.D.) show that the biological value of Kashmir soya bean, Lyallpur soya bean, green gram, dal arhar, and Bengal gram, (received this year) are 43, 57.7, 43, 62 and 63 respectively, while corresponding digestibilities are 94.36, 95, 94, 92 and 94. These experiments are being repeated on another human adult (I.B.G) and results so far obtained show that biological values for Lyallpur soya bean, dal arhar and Bengal gram are 58, 62.8 and 63 respectively.

14. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. V. N Patwardhan at the Seth G. S. Medical College, Bombay.

As stated in the previous summary report for the year 1941-42, the assay of the biological value of the soya bean protein as compared with the other pulse proteins, namely, dal arhar, Bengal gram and green gram by metabolic experiments and growth tests, has been completed. The results of the same are given in table I.

TABLE I.

Biological value by metabolic experiment and growth test.

Name of pulse.	Protein level p.c.	Biological value.	Digestibility coefficient.	Total protein gm. p.c.	Available protein gm. p.c.	Biological value by growth test.
Soya bean variety I	9.75	57.5	91.4	42.19	22.17	0.50
Bengal gram	9.69	61.1	86.6	23.20	12.27	1.31
Dal arhar	9.87	64.8	86.2	23.67	13.22	0.82
Green gram	9.69	47.7	85.0	25.56	10.36	0.56

The supplementary value of soya bean and other pulses was also determined. The supplement being 3 oz. of each pulse to the diet given. The results are given in table II.

TABLE II.

Supplementary value of soya bean and other pulses (supplement of 3 oz. of each to a poor rice diet).

Name of pulses.	Average food intake per day. g.	Total gain in weight in 8 weeks. g.
Soya bean	4.8	11.3
Bengal gram	5.3	18.0
Dal arhar	5.3	17.2
Green gram	5.1	13.5

Three new samples of soya bean and three samples of pulses have been received and the assay of the biological value of these samples as compared with other pulse proteins by the balance method and growth tests is in progress. The results of these experiments are expected to be ready within the next few months.

15. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. Bashir Ahmad, at the University Chemical Laboratories, Lahore.

(1) Experiments on the biological value of proteins of soya bean, dal arhar, green gram and Bengal gram by (i) growth method and (ii) metabolism experiments has been concluded. The results are briefly as follows :—

	Growth method.	Metabolism Tests.
Soya bean (Manchurian) ..	.. 0.78	58.90
Dal Arhar ..	.. 0.70	73.60
Green Gram ..	.. 0.95	65.66
Bengal gram ..	.. 1.30	63.01

(2) The effect of baking and autoclaving on the biological value of soya bean protein and Bengal gram protein has been determined.

GROWTH METHOD

Soyabean :

(i)	Baked	(a) 0.71—June, July experiments. (b) 1.10—September, October experiments.
(ii)	Autoclaved	1.2

Bengal gram :

(i)	Autoclaved	1.77

Experiments conducted during hot whether on Lyallpur variety of soya-bean and metabolism tests on various pulses under different heat treatments, gave very inconclusive results and therefore are not being reported. With the coming of the cold weather, the experiments on the effect of cooking are all being repeated.

(3) The effect of supplementary value of pulses, when added to a poor rice diet has been studied with the following results :—

	Average increase in weight per week.
Poor rice diet alone ..	.. 0.75 gms.
Poor rice + soya bean ..	.. 1.31 „
Poor rice diet + Bengal gram ..	.. 2.03 „

Those experiments were also conducted during the hot weather.

(4) A human experiment was conducted on school children to study the effect of soya bean and dal mash on their rate of increase in weight, height and chest measurement. All the children belonged to a class of low economic level, and were vegetarians. Measurements were taken at the beginning of the experiment and again after a period of 7 months. A large increase in height was

obtained with both soya bean and dal mash feeding ; that of soya bean being larger. The result was statistically significant. An increase in weight was also obtained in the treated groups but the figures were not statistically significant. The effects on chest measurement were also not of any statistical value.

16. Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmed at the University Chemical Laboratories, Lahore.

The object of this enquiry was to investigate into factors responsible for abnormalities of the calcium and phosphorus metabolism of which there is a high incidence in Kangra and certain other areas of the Punjab. The investigation has been pursued along the following lines :—

Dietary factors.

The department of Punjab Public Health has carried out an extensive survey of the total calcium and phosphorus contents of the diets, and soils of the Kangra area. They have shown that these are highly deficient in both these minerals. No attention has however been paid to the available calcium and phosphorus, nor to the phytin content of the foods. It is well known that phytin has an important relationship to the availability of these minerals. Some 20 foodstuffs from representative areas of the district have now been examined for their available calcium, phosphorus and phytin content. It is found that the ration of available calcium and phosphorus is only 0.2. It is evident that such a ratio is highly adverse to the utilisation of both calcium and phosphorus.

Blood serum calcium, phosphorus and phosphatase.

It is recorded in literature that in rickets and osteomalacia blood phosphorus values are generally low and phosphatase high. Dietary studies now undertaken in the Kangra area show that the diet of the people is grossly deficient in calcium and relatively high in phosphorus. In view of this observation some hundred cases comprising of 10 normal adult males, 20 normal adult females, 25 normal children, 20 cases of rickets and 25 cases of mild and severe osteomalacia have been investigated for blood serum calcium, phosphorus and phosphatase. It has been observed that phosphorus and phosphatase are generally within normal limits for nomal healthy people investigated. Even for cases of rickets and osteomalacia the blood serum phosphorus varied within normal limits and phosphatase though slightly elevated did not show the phenomenal increase recorded by other workers. On the other hand blood serum calcium was somewhat low for normal persons and very low in pathological cases. The values for these ranged between 7.84 to 6.40 mg per 100 c.c. of blood serum. In one case a value as low as 6.2 mg calcium per 100 c.c. of the serum was recorded. These observations indicate that pathological conditions met with in this area are of the high phosphorus-low calcium type. Even clinical symptoms in the patients were such as have been recorded for high phosphorus—low calcium rickets in animals.

Organic phosphate esters of the blood.

In view of the fact that in rickets and osteomalacia a derangement of phosphorus metabolism had been commonly observed by other workers, it was decided to investigate in detail the various phosphorus fractions both in normal

and in pathological cases. These included preformed inorganic phosphorus, adenosine-triphosphate, hexosediphosphate, hexosemonophosphate, acid-soluble phosphorus, glycerophosphate, total phosphorus, lipid and nucleic acid phosphorus. The norms of each fraction have been tentatively established by an investigation of 25 normal cases. About 40 pathological cases including those of liver diseases, anaemias, malaria, dysentery have since been studied prior to those of deranged calcium and phosphorus metabolism. This has been done with a view to obtain the blood phosphorus pictures in such diseases which often complicate the mineral derangement. This work is yet in a preliminary stage.

17. Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipur, Patna.

Biological value of common pulses.

The enquiry was started late in the year.

Six adult human males in apparent good health and weighing from 40 to 56 kg were chosen as experimental subjects. They were kept on a low-nitrogen diet for a period of six days. The low-nitrogen diet consisted of a preparation of sago, sugar and ghee (butter oil) and biscuits made locally from corn starch, butter, sugar, honey, baking powder and table salt on the lines suggested by Sumber & Murlin (J. Nutrit., 1938, 16, p. 141). Small servings of boiled potato, bottle gourd and one particular type of leafy vegetable cooked in vegetable oil were also given. The approximate raw weight of the vegetables were 150 g, 100 g, and 100 g respectively per subject per day. The dry weight of the food taken daily varied from 800—1000 g and the weight of nitrogen varied from 0.31 g to 0.66 g. The subjects received vitamin supplements throughout the periods.

During subsequent test periods of six days each time, the subjects were given one kind of each of the common pulses red gram (*Cajanus indicus*) Bengal gram (*Cicer arietinum*), lentil (*Lens esculenta*) and green gram (*Phaseolus radiatus*) in addition to the foodstuff consumed during the low-nitrogen period. The diet consisted of nitrogen free biscuits, gruel made of the particular pulse under investigation, khichri made of sago and the pulse and the servings of boiled potato and leafy vegetable cooked in oil. The food was made as far palatable as possible. Except lemon no other vitamin supplement were given. In the test diets nitrogen supplied from foods other than pulses barley exceeded 20% of the total N intake and the proteins thereof provided 4.5% to 5% of the total calories in the test period. The stool and urine voided during the last three days of each experimental period were collected and the nitrogen content of the respective samples were estimated by the kjeldahl method. The calorie value of the diets during the low nitrogen period and the test period varied from 2800 to 3500 daily but almost constant in each case. The subjects performed their normal duties in the laboratory even during the low nitrogen period.

The average digestibility coefficient and the biological value of each of the pulses were calculated according to the formulae suggested by Chick *et al* (Biochem. J., 1935, 29, p. 1702).

The average digestibility coefficient of red gram was found to be 94.6 ; that of Bengal gram 90.8 ; lentil 92.4 and green gram 91.8. The respective biological value were found to be 89.3, 86.9, 85.1 and 82.9. The data are being analysed statistically.

At present feeding experiments have been started to find out the ratio of pulse protein : rice protein which would ensure the most economic utilisation of proteins of the four pulses enumerated above.

18. Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Mr. B.N. Banerjee at the Indian Institute of Science, Bangalore.

COMPARATIVE STUDY OF THE METHODS OF ESTIMATION OF VITAMIN A.

The Hilger Vitameter A has been found to give as accurate results as the quartz ultraviolet spectrophotometer especially if the average of a number of readings at different concentrations of the oil is taken. The unsaponifiable fraction of the oil gives a lower value than the whole oil showing that there is considerable irrelevant absorption.

The Pulfrich Photometer with filter S.61 has been standardised for measuring the intensity of the blue colour given by vitamin A with antimony trichloride. On the whole oil, the extinction coefficient is proportional to the concentration of the oil only below a value of 0.5 when a 1 cm. cell is used. The estimation on this instrument is more rapid and easier than on the Lovibond Tintometer. The unsaponifiable fraction develops a deeper colour than the whole oil.

Deterioration of the vitamin A in Shark liver oil under different conditions.— Accelerated oxidation tests were performed by storing 2 c.c. of the oil in flat-bottomed specimen tubes (2.2 cms. diameter) under different conditions. At recorded intervals two tubes were removed and individual determinations of peroxide number and vitamin A were made on the oil and the mean used for graphical representation.

Influences of temperature.—Experiments were carried out at room temperature (21°-24°C), 40°C., 50°C. and 100°C. in darkness.

At 100°C. there is no accumulation of peroxides but 50% of the vitamin A is destroyed in 10 hours. At 50°C. the peroxide number increases rapidly and the time—peroxide curve shows no inflexion characteristic of an auto-catalytic reaction. 50% of the vitamin A is lost in 6 days. At 40°C. there is a clear period of slow peroxide formation (period of induction) followed by rapid peroxide formation. The induction period is 4 days and 50% of the vitamin A is lost in 8.5 days. At room temperature the induction period is 8 days and 50% of the vitamin A is destroyed in 20 days.

Catalytic influence of metals.—Equal volumes of metal powders of uniform mesh size were kept in contact with 2 c.c. of the oils in an incubator at 40°C. Three tubes were removed periodically and the oil analysed, the average being taken for graphical representation. The metals tried may be listed in the following order of decreasing pro-oxidative activity: copper, mild steel, stainless steel, nickel, (tin, aluminium) and zinc. In the case of copper the peroxide number increased to a maximum and then fell down though the vitamin A continued to get destroyed at the same rapid rate. Tin and aluminium were almost equally active and zinc has the least harmful action. Hence galvanized vessels and containers are the safest to use in the shark liver oil industry.

Influence of inert atmosphere.—2 c.c. of the oil were taken in specimen tubes and the oil degassed under vacuum for an hour. After saturating with the particular inert gas, the tubes were corked tight and wax applied over the cork. These were stored at the required temperature,

In an atmosphere of nitrogen and at room temperature the samples lost 20% of the original vitamin A in 25 days. At 40°C. the loss was 50% in the same time. In an atmosphere of carbon-dioxide at 40°C. The destruction was 30% in the same period. Hence storage under an inert gas is very useful in preserving the vitamin potency.

Influence of antioxidants.—Three samples of commercial antioxidants supplied by the Mysore Vegetable Oil Products Ltd. were tested and one of them has been found to be very promising.

19. Enquiry into the relationsnip between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The work during the year was restricted to only two members of the vitamin B complex namely Nicotinic acid and Riboflavin. The tumours under investigation were hepatomas in rats produced by feeding Dimethyl-amino-azobenzene (Butter yellow) and intra-oral cancer in hospital patients.

Animal experiments.

Four batches of albino rats with the necessary number of controls were kept on a diet deficient in vitamin B complex. They were fed 4 mgms. of butter yellow in olive oil for periods ranging from 45 days to 150 days. It is intended to kill these animals after varying intervals and to study progressively their liver metabolism as regards nicotinic acid and riboflavin. This experiment is in progress.

Observations on hospital patients.

Martin and Koop (1942)—on the basis of their clinical observations—have suggested that precancerous mouth lesions are probably caused in man by avitaminosis B. It was therefore thought worth while to examine the patients admitted to the hospital with oral cancer and to determine the state of saturation of such patients with nicotinic acid and riboflavin. Saturation tests are carried out after administering the vitamins orally as well as by intravenous injections. So far, ten patients have been studied and from the results obtained it appears that the state of saturation with these vitamins is very poor in intra-oral cancer patients. The study is in progress.

20. Enquiry to organise research into the best methods of treating starvation cases, under the Director, All-India Institute of Hygiene and Public Health, Calcutta.

Origin and object of the enquiry.

The Nutrition Advisory Committee at its meeting on 1st and 2nd October recommended a scheme of investigation into the methods of treatment of starving destitutes that were being admitted into the hospitals. The Govern ng Body sanctioned a sum of Rs. 11,100/- to carry out the work for a period of approximately six months. Preliminary studies were already started from the later part of August and paid staff of workers were employed from 19th October.

Distribution of work.

Work was immediately undertaken at the following hospitals and laboratories.

1. Campbell Hospital.
2. Dr. Suresh Sircar A.R.P. Hospital.
3. Lee Memorial A.R.P. Hospital.
4. Dept. of Applied Chemistry, University College of Science.
5. Indian Institute of Medical Research.
6. School of Tropical Medicine.
7. All India Institute of Hygiene and Public Health.

Picture of starvation cases.

Most of the cases admitted were emaciated showing low Systolic, Diastolic and pulse pressure, hypoproteinemia, hypoglycemia and a certain degree of anemia. A number of such cases were in a state of collapse. Many of the patients were also suffering from fevers, diarrhoea and dysenteries. The clinical conditions associated with vitamin deficiencies were conspicuous by their absence. The death rate among sick destitutes admitted into hospitals were very high. To enable an intensive study into the cause of this high death rate the Director of School of Tropical Medicine placed at the disposal of the enquiry one male ward of 18 beds in the Carmichael Hospital for Tropical diseases. It was now realised that diseases such as Malaria, Dysenteries, Pneumonia were responsible for the high death rate. The observations were however made on a small number of cases.

The bearing of disease in malnutrition.

To find out the incidence of Malaria and Dysenteries among the large number of sick destitutes in other hospitals investigations were done on a large scale on several hundreds of cases admitted into the Behala A. R. P. Hospital and the Campbell Hospital. This confirmed the previous view that the treatment of these cases of sick destitutes was not altogether a nutrition question and that a more complete picture of the clinical background of the cases and the existence of intercurrent diseases should be borne in mind.

Clinical types.

The following clinical types were recognised among the sick destitutes :—

1. Inanition.
2. Fevers
   - Malaria
   - Pneumonia
   - Unclassified
3. Intestinal Disorders—
   - (i) Bacillary Dysenteries
     - Shiga
     - Flexner
     - Schmitz
   - (ii) Amaebic Dysentery
   - (iii) Cholera
   - (iv) Unclassified
4. Oedema
   - (i) Nutritional
   - (ii) Nephritis.
5. Anemia

6. Skin conditions

(i) Ulcers. Naga Sore.
(ii) Scabies.

LABORATORY FINDINGS.

The laboratory investigations consisted of the following examination.

1. BLOOD.

(a) *For malaria parasites.*

This work was done mainly by the Department of Protozoology, School of Tropical Medicine. Over two thousand blood films were obtained at random from a large number of sick destitutes and were found to be positive for malaria in over 32% of cases. Of these malignant tertian infection formed 20%, benign tertian 12% and quartan less than 0.5%. Mixed infection of M.T. and B.T. were noted in about 2% of the cases.

(b) *Biochemical examinations.*

These were done in the following laboratories.

1. Campbell Hospital, Physiology Department.
2. University College of Science, Applied Chemistry Department.
3. School of Tropical Medicine, Biochemistry Department.

Examinations were made of Albumin, Globulin, Non-Protein-Nitrogen, Ammonia, Glucose, Chloride, Uric acid, Greatinine, Cholesterol, Lecithin, and Calcium. Some two hundred samples of blood were examined. Albumin, Glucose, Uric acid and Cholesterol were found to be reduced in almost all cases. Chlorides and Calcium were found to be within normal range. Increase over normal values were observed in the cases of Globulin and Lecithin. Glucose tolerance tests were done in a number of cases both by oral and intravenous methods. A majority of these cases indicated a definite breakdown of carbohydrate metabolism both in utilisation and storage of sugar.

(c) *Haematological investigations.*

This was done mostly by the Haematology Department of the School of Tropical Medicine. Complete cytological examinations were done on over 70 cases and Haemoglobin estimations in about 300 cases. Over 80% of the cases showed some degree of anaemia of which 37% were moderately severe and 16% were severe. More than 70% of the anaemias were of Macrocytic type.

The estimations of blood volume and specific gravity of blood did not show any haemoconcentration even in very emaciated cases.

2. EXAMINATION OF STOOLS.

This was done in the Department of bowel-diseases and protozoology, School of Tropical Medicine and the Indian Institute of Medical Research. About 350 samples of fresh stools were examined both microscopically and culturally. Specific Dysentery organisms were isolated in about only 20% of the cases of which over 16% were B. Flexner and about 2% B. Shiga and over 1% were B. Schmitz, while over 50% of the specimens showed on microscopic examination cellular exudate suggestive of some Bacillary infection. Entomaeba histolytica was found in about 9% of the cases.

3. Examination of urine.

This was done mostly in the Biochemistry Department of the School of Tropical Medicine.

The quantity passed in 24 hours was always very small being as low as 300 to 600 c.c. in most of the cases. A trace of albumin was a constant finding. Chloride and urea excretion in 24 hours were low in most cases.

4. Examination of gastric juice.

This was done in the Department of Haematology, of the School of Tropical Medicine.

Fractional gastric analysis was done in 30 cases and of these 9 cases did not show any free acid either in the fasting juice or post parandial period.

5. Examination of bone marrow.

This was done in the Department of Haematology, of the School of Tropical Medicine.

Sternal biopy was done in 20 cases and in only 4 cases the marrow was hypoplastic to red cells.

6. Examination of smears from ulcers.

This was done in the Protozoology Department of the School of Tropical Medicine.

Smears from ulcers in over 60 cases were examined microscopically. Fusiform bacilli were found in over 61% of the cases and fusiform bacilli and spirochactes in over 22% of cases. Smears were also examined from two cases of cancrumous. Fusiform baccilli and spirocbeates were found in both the cases.

7. Post mortem examination.

Post mortems were done in the Campbell Hospital and the School of Tropical Medicine and Histopathology of the material obtained is being worked in the section of Biochemistry and Nutrition, All-India Institute of Hygiene & Public Health.

The main pathological findings were as follows.

Extreme emaciation and reduction in the size of the various organs with loss of fat in all parts of the body. Dilation of right side of heart, passive congestion of viscera and clear exudate in cavities were found in most cases ; thinning out and denudation of the mucous membrane of the intestines were noted in over 80% of the cases. Pathological conditions associated with malaria, dysenteries and tuberculosis of the lungs were found in a number of cases.

Treatment.

The treatment of the sick destitutes was considered from the two aspects of nutrition and disease. Detailed instructions regarding this was embodied in a pamphlet prepared by a sub-committee. The main features in this pamphlet regarding the treatment of the more commonly found conditions are :—

1. Inanition treatment by protein hydrolysates with glucose.

2. *Malaria* :—Oral administration of quinine is usually effective. Mass treatment with oral quinine is recommended in endemic areas and during malaria seasons.

3. *Dysenteries*:—All cases to be treated as bacillary with sulphaguanidine unless there is definite indication to the contrary.

4. The doses of drugs for diseases in starvation cases should approximately be half the usual therapeutic doses.

The diets in these cases should be gradually worked up from liquid diet in early stages through milk and milk products into gruels containing cereals. Special attention should be paid to provide a liberal supply of vitamins and minerals though signs of deficiency diseases are not present.

Proposed plan of work.

It is proposed to continue the work on clinical and laboratory investigations on lines already indicated. Recent observations show an increase in oedemas of non-nephritic origin. Investigations are being undertaken on these cases.

Some of the cases die after a few days' stay in the hospital in spite of absence of any disease condition and inspite of best nursing, dietetic and medical help. It is proposed to investigate the best method of management of these cases.

It may be pointed out that most of the work was carried out by or under the direction of a large number of willing voluntary workers attached to various hospitals and institutions in Calcutta. The methods of treatment have been worked out and practised in the Campbell Hospital and Carmichael Hospital for Tropical diseases.

The results obtained from these investigations have been helpful to the Government in setting up a large number of hospitals all over the province for the treatment of sick destitutes.

LEPROSY

21. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

IMMUNOLOGICAL STUDIES.

(a) *Improved technique of obtaining the antigens from Hensen's bacillus* :

Previous work under this enquiry had given clear indications of the existence of a specific antigen of the leprosy bacillus, and had encouraged further attempts to isolate it in a more natural form.

The isolation of leprosy bacilli from leprous nodules by the chloroform method developed in this laboratory involves the use of heat, the nodules being autoclaved before extraction with chloroform, and the chloroform extract being evaporated over a water bath. The use of heat, specially the autoclave temperature, is very likely to change the nature of the antigen, and may be responsible for the observed lack of specificity of the resulting antigen. It was therefore decided to try to isolate the bacilli without the use of heat at any stage in the process. Such a technique has now been evolved. Instead of being autoclaved before extraction with chloroform, the nodules are frozen in a mixture of CO_2 snow and methyl cellosolve ; and instead of being evaporated over a water bath, the chloroform extract is evaporated in vacuum at room temperature. Nucleo-protein has been prepared by the phosphate buffer method from the bacilli isolated by the above technique.

It was expected that the reaction produced by the nucleo-protein obtained by this method will be more specific than that produced by the nucleo-protein obtained from the heat-treated bacilli. However, the results of the tests in non-contacts in the Punjab with this fraction have not come up to expectation. It appears that the chloroform used for extracting the bacilli is not completely removed in the cold process, and that the traces of chloroform left behind interfered with the results.

(b) *Antigenic elements of other acid-fast bacilli* :

A study has been made of the antigenic elements of the other acid-fast bacilli, in the hope that antigens suitable for work in leprosy may be isolated from these other bacilli. During the year the study has been completed of the Kedrowsky's bacillus, and the Stefansky's bacillus (the causative organism of rat leprosy). The Kedrowsky's bacillus was obtained from a broth culture of this organism ; the Stefansky's bacillus was obtained from tissues of infected rats by means of the chloroform method used for obtaining the Hansen's bacillus from leprous nodules.

Four fractions, similar to the fractions from Hansen's bacillus, have been isolated from the two acid-fast bacilli studied. These fractions are :— the polysacchride, the nucleo-protein, the acid-soluble protein, and the alcohol-soluble protein.

The following table gives the results of skin tests with the various fractions in patients of leprosy of the two types :—

Fraction	Hansen's baccillus		Kedrowsky's bacillus.		Stefansky's bacillus.	
	N Cases	L Cases	N Cases	L Cases	N Cases	L Cases
Polysaccharide	—	—	—	—	—	—
Nucleo-protein	+	—	+	+	+	+
Alcohol-soluble protein	+	—	+	+	+	+
Acid-soluble protein	+	—	+	+	+	—

N—Neural. L—Lepromatous.

It will be seen from the above table that like the similar fractions of the Hansen's bacillus, the polysacchride fraction of these bacilli is inactive, while the three protein fractions are active. The results further indicate that of the three protein fractions the acid-soluble fractions from the two bacilli, appear to be similar antigenically to the similar fraction of Hansen's bacillus. All the three protein fractions of the Hansen's bacillus produce reactions in the neural cases only. Of the protein fractions of the other acid-fast bacilli only the acid-soluble fraction behaves similarly, the other two fractions producing reactions in both the neural and the lepromatous cases. This line of work seems to be very promising. It appears that it would be possible to isolate, from the culture of some of the acid-fast bacilli, a fraction which is similar to the lepromin prepared from Hensen's bacillus in its action in cases of leprosy, contacts and non-contacts. This would very much facilitate the preparation of 'lepromin' in large amounts. At present the only source of lepromin is the leprous nodules removed from the patients.

In addition to the skin tests the various fractions of the Kedrowsky's and Stefansky's bacilli have been used for doing precipitin tests with sera of cases of leprosy of both types. Such tests have always given negative results irrespective of whether the skin tests with the fraction tested were positive or negative.

Clinical studies.

(a) *Cases with atypical lesions* :

A study is being made of patients of leprosy with lesions which are not typical of either type of the disease, with a view to correlating the clinical, histological and immunological findings in these cases with the prognosis of the disease.

(b) *Ill-defined lesions in the neuro-macular cases* :

A study is being made of the bacteriological, histological and immunological findings in neuro-macular cases of leprosy with ill-defined lesions, with a view to correlating these findings with the prognosis of the disease in these cases.

(c) *Neural lesions in children* :

A study is being made of the prognosis of the disease in children with the different kinds of neural lesions (simple, tuberculoid etc.).

Therapeutic studies.

(a) *Sulphanilamide derivatives* :

A trial has been made of the therapeutic value of an emulsion in arachis oil of a sulphanilamide derivative injected into the bacteriologically positive lesions of leprosy. 2 c.c. of the emulsion containing 2 grains of the drug was

injected intradermally into one part of a lesion, a similar quantity of plain arachis oil being injected into the other part of the lesion. Four to six injections were given, spread over a period of two months. Repeated smears taken from the lesions showed that the drug in the doses used had no bactericidal action on the leprosy bacillus.

(b) *Substitute for trichlor-acetic-acid* :

Trichlor-acetic-acid, used as a local treatment for painting the lesions of leprosy, is now-a-days difficult to obtain. Local application of carbolic acid was tried as a substitute and it was found that a 1 in 5 dilution of this acid gave the same result as a similar dilution of the trichlor-acetic-acid There is, however, one disadvantage, that is, carbolic acid does not mix well with water. The mixture should therefore be shaken thoroughly before use.

(c) *Substitute for creo sote*:

The hydnocarpus oil used for injection is usually mixed with 4% creosote. The double distilled wood tar creosote used for this purpose is now unobtainable in the market. Various substitutes for this have been tried. The addition of 1% thymol to the oil has been found to be very satisfactory but the oil containing thymol has not been used long enough to be sure that its prolonged use produces no unpleasant effects. Various fractions of a sample of crude coal tar creosote are being investigated regarding their suitability for mixing with the oil.

ANIMAL EXPERIMENTS.

(a) *Infection of splenectomised monkeys* :

Six splenectomised monkeys were twice inoculated with human leprosy material over 3½ years ago All the injected monkeys remained well for more than 3 years. During the present year three of these monkeys have died and the other 3 have been sacrificed. In none of them was there found any macroscopic sign of leprosy, and smears made from the various organs were negative for acid-fast bacilli.

(b) *The bactericidal effect of sulphanilamide drugs on rat leprosy bacillus in vivo* :

The bactericidal effect of sulphanilamide drugs *in vitro* on rat leprosy bacillus was investigated last year. It was reported that sulphapyridine and sulphanilamide, in a dilution of 1/1000, possess a bactericidal effect *in vitro* on the bacillus of rat leprosy if allowed to act on the organism at 37°C for 48 hours. A study has now been made of the effect of these drugs on this bacillus *in vivo*. The results have not been encouraging, since the oral administration of M.B. 693 in doses that could be tolerated by the rats has not prevented the development of generalised infection in rats infected with rat leprosy material.

(c) *Diet and susceptibility to experimental rat leprosy* :

Recently a worker in South India had stated that well-fed white rats kept in good climate are immune to experimental infection with rat leprosy bacillus. With the collaboration of the Director of the Nutrition Research Laboratories, Coonoor, an experiment was undertaken to investigate this matter. Infected rats were taken from Calcutta to Coonoor, where an emulsion from the infected tissues of these rats was made. Healthy white rats were injected with this emulsion, intraperitoneally and intramuscularly, and were kept at Coonoor on the stock diet of that laboratory. All the rats surviving for more than four months after the injection developed generalised rat leprosy. The results of this experiment therefore do not lend support to the statement that well-fed rats living in good climate are not suspectible to experimental rat leprosy.

Culture work.

Attempts to grow rat leprosy bacillus in media containing the product of broken down bacilli have been continued. There is some evidence of multiplication and subcultures are in progress.

Epidemiological studies.

(a) The epidemiological studies at the Bankura Investigation Centre have been continued. The findings made during the last six years have been received. Following is a summary of the main findings :—

(i) The incidence, type distribution and age distribution of cases of leprosy have shown little change during the six years period under review. In spite of the absence of any effective isolation measures there is no evidence that leprosy is increasing in the area. In this area as in other similar areas statements have frequently been made that leprosy is rapidly increasing. This study does not lend support to this idea.

(ii) Definite evidence of contact was obtained in 56 (66%) of the 85 new cases that have arisen in the area during the six years. This illustrates the difficulty of tracing sources of infection even in a population which is constantly under observation.

(ii) Eight neural cases have become lepromatous during the 6 years. It appears that the lesions of the neural type, most likely to change later into the lesions of the lepromatous type, are the rather indefinite flat patches with little or no definite sensory change, appearing in children with a negative lepromin reaction.

(iv) All the 8 neural cases that have become lepromatous were lepromin negative when tested before the change. This demonstrates very clearly the prognostic value of the lepromin test in cases of leprosy of the neural type.

(v) Of the 23 new lepromatous cases 15 (65%) have been lepromatous from the start. This and other findings made in the area are not in accordance with the view that leprosy usually starts as a neural type and later passes on to lepromatous type.

(vi) It appears that suspicious patches in children living in close contact with infectious cases are of far graver import than similar patches in children with no such contact.

(vii) The members of families with lepromatous cases are not only more likely to get the disease, but they are also more likely to get it in a serious form, than the members of the families with a neural case or no case of leprosy. The one obvious factor contributing to this greater susceptibility is the greater chance of close and prolonged contact with infectious cases. It is possible that familial differences in susceptibility also contribute towards this.

(b) Dr. Isaac Santra working under the Indian Council of the British Empire Leprosy Relief Association has continued accurate leprosy surveys of the whole population of selected small areas. This year he has worked in two areas in Bihar and in one area of Bombay. The findings made in Bombay are not yet available. The main findings of the surveys in Bihar are summarised in the following table :—

Area.	Population examined.	Incidence %	Type distribution. % I. cases	Age distribution. % aged 15 & under.	Sex distribution. % male.
Chirkunda (Manbhum Dist.)	5192	3.02	14.64	18	52
Hiranpur (Santal Pargs.)	4991	2.06	10.6	11	53

The following comments may be made :—(1) Although the incidence is fairly high, the proportion of lepromatous cases and the proportion of cases in children below the age of 15 are comparatively low. (2) The number of cases in the two sexes is about equal. It is generally believed that leprosy is much more common among men than among women, usually in the proportion of about 2 to 1. Findings similar to the ones made in the two areas of Bihar, have also been made in some recent surveys in other parts of India. This would indicate that the marked disparity in the incidence of the disease in the two sexes is not always to be found.

22. Leprosy enquiry under Dr. R.G. Cochrane, Chief Medical Officer, Lady Willingdon Leprosy Sanatorium, Chingleput, Madras.

The work of this enquiry has proceeded as in former years along the following lines :—

(1) Epidemiological.
(2) Clinical.
(3) Pathological and Immunological.
(4) Animal experiments.
(5) Therapeutic experiments.

Epidemiological studies.

This work continued in two directions ; the study of the various incidence of leprosy in relation to age and type distribution of cases, and re-survey where possible of areas already surveyed. This year the Adi-dravida colony of Saidapet was due for a re-survey, but unfortunately owing to the recent floods the whole village was swept away. The project therefore, had to be abandoned. The second direction along which epidemiological work is being pursued is the relationship between contact and the development of the disease.

With regard to new surveys, the most interesting of these is a group o villages in the N. Arcot District. The following table gives the summary of the findings and these figures are compared with findings of some of the previous surveys :—

TABLE I.

Village.	Gross incidence.	Child incidence.	Child rate.	Open case rate.
	per cent.	per cent.	per cent.	per cent.
Mottur	12.40	14.94	33.30	30.77
Gummakuppam	8.36	8.68	44.20	32.70
Gummakuppam Cheri	5.81	3.74	41.10	29.40
Kosavanputhur	4.38	3.80	34.10	45.45
Area 'A'	6.71	8.85	48.80	24.80
Area 'B'	6.04	8.30	47.64	16.70
Fenpet	2.54	2.85	40.00	26.60
Eddapalyam	3.50	2.07	21.90	12.50
Mambalam village	2.98	2.96	42.80	14.30

The survey figures are analysed according to the survey report of the I. R. F. A. Two things reveal themselves in an analysis of the findings. First, that so far no relationship can be discovered between the incidence in the various age groups and the gross incidence of the disease. Secondly, in villages of comparatively high incidence the child incidence and child rate tends to be high. This is especially so in the case of the child rate in villages where the incidence is above 50 per thousand. Many factors influence the child rate; for instance, in Mambalam village (Saidapet Adi-dravida colony) the child-rate is high and the incidence low. This is explained by the fact that in Mambalam village the contacts of every open case were traced and several children in the houses were infected by the same open case, and in Fenpet the figure is high because one open case infected five children. It is of utmost importance in the planning of a leprosy campaign to be able to state whether the disease is of important significance in a given area. Although the criteria for this have not yet been definitely discovered, it can be taken as a general guide that where the child rate is high, and if this is associated with a high child incidence the disease appears to be of greater severity. The answer to the question " What constitutes a serious infection of leprosy in a village ?" may be found in the distribution of the disease according to age groups. The following table gives this comparison.

TABLE II.

Comparison of incidence in various age groups.

	VILLAGES.										
	Mottur	Gumma-kuppam	Gumma-kuppam Cheri	Kosavan-pet	Saidapet Area A	Saidapet Area B	Fenpet	Edapa-layam	Mambalam	Peram-bakkam Cheri I. 1942 June.	Peram-bakkam Cheri II. 1943 Dec.
Gross incidence	124 per 1000	83.6 per 1000	58.1 per 1000	43.8 per 1000	67.1 per 1000	60.4 per 1000	25.5 per 1000	35.0 per 1000	29.9 per 1000	60.4 per 1000	83.6 per 1000
Age group											
0—14	33.3%	44.2%	41.1%	34.1%	54.72%	47.6%	40.0%	21.9%	40.62%	50.0%	36.36%
15—34	36.0%	30.8%	17.8%	27.3%	26.42%	24.7%	20.0%	34.4%	28.13%	12.5%	27.28%
Over 34	30.7%	25.0%	41.1%	38.6 %	18.86%	27.7%	20.0%	43.7%	31.28%	37.5%	36.36%

It will be noted that in all the areas of high incidence the percentage of cases in the younger age groups is as high or higher than in the older age group. In Eddapalayam where the incidence is comparatively low the percentage of cases in the over 34 age group is markedly greater. Two figures are of interest, the incidence in Fenpet area and the results of the survey in Perambakkam in June 1942 and December 1943, 18 months later. The increase in the percentage of cases in the 0—14 age group in Fenpet may be artificial for one open case infected five children in his own family. In the 1942 June survey in the Adidravida Colony of Perambakkam the incidence is 60.4 per thousand while the incidence in the 1943 December survey has risen to 83.6 per thousand and yet the percentage of children in the 0—14 age group has dropped from 50% to 36.36% In this period all open cases had been under partial or complete isolation and the three newly discovered cases were all adults of the neural type, one a new arrival and the other two old inhabitants possibly missed in the previous survey. It would be of interest to discover whether a marked alteration in the percentage of cases in the higher age group indicates a less serious situation and possibly a tendency for the disease to decrease in a village.

The study of the relationship between contact and the development of the disease has been continued, and an up-to-date analysis of these figures confirms our previous findings that children most readily acquire leprosy from intrafamilial contact, and that the seriousness of leprosy has a direct relationship to the intensity of contact. Further, a greater number of cases have been traced to room contact than house contact. These findings indicate that the spread of leprosy is closely related to that of tuberculosis, and that the disease is a room rather than a house infection. This finding particularly applies to urban areas. There are several factors in rural areas which may cause modification of this statement.

Clinical studies.

A study of the development of lesions of leprosy is of utmost importance and has a bearing on the epidemiology as well as the progress of the disease. In a further analysis of the figures the impression already gained is strengthened, and that is, that the lesions most likely to become lepromatous are the simple macules of neural leprosy and incipient lesions of children. So far, only 3 clinically tuberculoid cases, all of the minor variety and lepromin negative have become lepromatous. With regard to the incipient lesions we are aware that the existence of this group as a definite clinical entity is doubted, but we ourselves believe that unless this group is recognised an important and potentially serious form of the disease will be left undiagnosed. The group is quite definitely separated clinically from the category known as 'suspicious'. It is interesting to note that of 118 suspicious cases 19 have so far developed definite signs of leprosy, indicating that the first lesion of leprosy is a macule, and that it is not always possible to say whether such macules are leprotic or not. It is of the utmost importance to be able to separate this suspicious group into macules likely to develop definite clinical signs, and macules not characteristic of leprosy. This study is being pursued.

Pathological and immunological studies.

Detailed histological studies have been continued throughout the year and the macules of leprosy can generally be divided histologically into the following:—

(a) Those that show indefinite and non-specific histology.

(b) Those which show definite tuberculoid histology.

(c) Those which show definite leproma histology.

(d) A large group in which the intermediate or border line cases are included, but which vary from what may be termed typical intermediate lesions to lesions with atypical tuberculoid histology and atypical leproma.

The first group can be divided into those which show non-specific cellular changes but with evidence of commencing nerve involvement, and those with no such involvement. In the intermediate group are included at present all lesions in which the features are not characteristic of tuberculoid leprosy or leproma, and it is believed that here will be found the explanation of anomalous findings of certain workers who report tuberculoid and lepromatous leprosy in the same sections. Our histological studies so far undertaken have confirmed the opinion previously held that true tuberculoid leprosy does not become leproma, and that every case where there is mixed tuberculoid and leproma histology a careful study will show that the case probably belongs to this intermediate category, the sub-types of which have not been completely elucidated. The study of this latter group is of great importance because many of these cases show remarkable improvement even without treatment, and therefore a mistake in classification would cause, and has caused, great confusion in assessing the value of therapeutic remedies.

A detailed study of leproma has been started. It will take some time to complete, but from a preliminary investigation of histological and clinical material, it is considered that there are various sub-types of leproma, a study of which may give a clue as to which lepromatous cases have a favourable prognosis. At present lepromatous histology is in somewhat of a confusion.

Immunological studies.

Routine lepromin tests are done on all cases in the Sanatorium, generally speaking, the refined Calcutta lepromin is used. Lepromin according to the Calcutta method has been prepared at the King Institute, Guindy. A comparison of this lepromin and that obtained from Calcutta shows that the former is slightly weaker, but the general reading of the results are not affected. It is confirmed that a positive lepromin has a definite relationship to prognosis and that the atypical cases usually have atypical lepromin findings. Therefore before any conclusions can be drawn that major tuberculoid lesions become leproma two conditions must be fulfilled. (1) Lepromin should be definitely positive, (2) Histology should be unequivocal.

No lepromatous case has as yet revealed a positive lepromin. A study of the intensity of the reaction confirms the Calcutta findings that there appears to be a relationship between the severity of the tissue reaction and the lepromin reaction in tuberculoid cases, but there is apparently no such relationship in intermediate cases where even when there is intense tissue reaction a lepromin result may be negative. 27 intermediate cases have been included in the lepromin series, all of which have been confirmed histologically. Sixteen showed a negative lepromin, 5 showed a weak positive, and in 6 the reaction could be described as a definite positive but none showed a strongly positive reaction.

Animal experiments.

This work with the co-operation of the Director of the King Institute, Guindy, has been continued during the year. There are now eight animals in

the experiment. The 8 animals in this series are divided as follows :—One animal splenectomised and inoculated with leprosy material and given daily injections of old lepromin. The other animal inoculated but not splenectomised and given daily injections of old lepromin. Two un-inoculated animals, one splenectomised and the other not-splenectomised are used as controls and are given daily injections of old lepromin. Both these experiments are repeated on another series of 4 animals in which the refined lepromin of Dharmendra is used, the King Institute preparation being employed.

THERAPEUTIC EXPERIMENTS.

The work on the optimum dose of the hydnocarpus remedies has been pursued. In previous experiments there appeared to be little difference in the results of treatment whether high dosages (above 15 c.cs.) or low dosages (below 10 c.cs.) were given. In this series three groups were chosen (A) on subcutaneous injections alone (B) on intradermal and subcutaneous, (C) on intradermal alone.

It will be seen from Table III that the number of cases which have become negative in the intradermal and subcutaneous group exceeds the number of negatives in those receiving intradermal injections or subcutaneous injections alone.

TABLE III.

Negative.	Much improved.	Improved.	Stationary.	Worse.	Total.
		Intradermal Group.			
4 3.5%	13 11.4%	29 26.8%	39 34.2%	29 26.8%	114
		Subcutaneous and Intradermal Group.			
15 13.0%	26 22.5%	24 20.8%	30 26.0%	20 17.0%	115
		Subcutaneous Group.			
2 3.9%	7 13.5%	2 3.9%	20 39.0%	20 39.0%	51

This may indicate that to obtain the best results in treatment it is necessary to use both the intradermal and subcutaneous methods. This opinion is strengthened as it is seen in Table IV, that the average length of treatment in the subcutaneous and intradermal groups was shorter than in the other two groups although the results were more satisfactory.

TABLE IV.

AVERAGE PERIOD OF TREATMENT

	Subcutaneous and Intradermal group.	Intradermal group.	Subcutaneous group.
Negative	2 years 1 month	3 years 1 month	3 years 10 months
Much improved	1 year 10 months	2 years 2 months	2 years 8 months
Improved	1 year	1 year 6 months	2 years

Therapeutic experimentation in leprosy are extremely difficult, and before any definite conclusions can be drawn this experiment must be continued for a further period.

It has been said from time to time that lepromatous leprosy may become spontaneously healed. In the present stage of our knowledge we do not feel justified in not treating moderately advanced leproma, but a small series of cases was taken all of which were early lepromas and which showed a few negatives among the smears. These cases were designed for convenience-partial positive. In the treatment group 3 became negative and one worse. In the no treatment group consisting of 6 cases 2 became negative and 3 worse. This confirms the impression that certain early lepromas do become negative without treatment.

Leprosy and syphilis.

Muir (1927) stated that a positive W. R. reaction in leprosy was most likely to be due to complicating syphilis. If this is the case then all patients with leprosy should show an improvement in the W. R. reaction on treatment for syphilis. The treatment used for convenience was that recommended by Muir Hg. 33. Ten cases were chosen for a preliminary experiment ; five were treated with Hg. 33, and five left as controls. In the control group two cases, both with strongly positive Wasserman and Kahn reactions, became negetive and in the treatment group none. The negative result corresponded with marked improvement in the clinical and bacteriological condition and suggests that leprosy was the factor which caused the positive reaction.

Rural unit.

The experiment with regard to night segregation has continued. In one small village 100% of the open cases have been segregated and have slept regularly every night in the segregation houses. In the other three villages the percentage has not been quite so high. It is difficult as yet to say whether the experiment in night segregation is succeeding. It is of interest to note that in the village where all the infective patients are completely or partially segregated no new child cases or infective cases have arisen in the last 18 months. Further the improvement in the clinical condition shows an appreciable difference in those cases attending regularly for treatment and segregation than in those cases who have not so attended. Table V gives the result.

Table V.

	Negative.	Much improved.	Improved.	Stationary.	Worse.	Total.
Regular treatment and segregation.	2	5	Nil	1	1	9
Irregular treatment and no segregation.	Nil	1	1	3	4	9

While the evidence is not conclusive it can be stated that the results both in segregation and treatment are encouraging and make us feel that this experiment is worth continuing, for if a simple method of segregation can be devised the control of leprosy in a rural area becomes possible of achievement.

PLAGUE.

23. Plague researches under the Director, Haffkine Institute, Bombay.

I. Plague vaccine.

1. *The relationship of the virulence of a strain to the protective power of the resultant live vaccine.*

Last year, it was reported that a live vaccine had a higher protective power than a killed vaccine. But, it was found that the strain which yielded a potent vaccine was only relatively avirulent. Such strains still killed mice when a sufficiently large dose was given. Investigation was started to determine the relationship between the virulence of a strain and the protective power of the live vaccine made from it. Strain 53/H was used and was made progressively avirulent by repeated subcultures at 37°C. Earlier results were reported last year and this work was continued during the year.

Subculture of strain.	Virulence measured as M.LD. for a mouse.	Immunogenic value measured as mouse protective dose.
25th	300,000,000	875 organisms.
31st	600,000,000	3,850 organisms.
36th	1500,000,000	11,250 organisms.
40th	2000,000,000	13,500 organisms.
50th	3000,000,000	15,700 organisms.
65th	Ceased to produce infection.	No protective power. (200,000,000 organisms failed to protect.)

It is to be noted that 4 to 5 million killed virulent organisms protect a mouse. It is, therefore, evident that if a live vaccine is to be prepared, a strain must have a certain minimal amount of virulence and power of invasiveness.

2. *Agar-grown and broth-grown vaccines* :

It was proposed to work with casein hydrolysate broth, but unfortunately, the necessary chemicals could not be procured to work with. The chemicals needed were ordered but have only just arrived. In the absence of that, nutrient broth had to be used and the following results were obtained :—

A. *Tests on Mice.*

Vaccine.	Mouse Protective dose.
(1) Nutrient broth vaccine incubated at 28°C for 3 days and at 37°C for 5 days—heat killed (Approx. 1,000 million organisms per c.c.)	0.005 c.c.
(2) Nutrient broth vaccine incubated at 28°C for 3 days and at 37°C for 5 days—killed with phenyl mercuric nitrate (0.0003%)	0.004 c.c.
(3) Nutrient broth vaccine incubated at 37°C for 8 days—heat killed.	0.004 c.c.
(4) Nutrient broth vaccine incubated at 37°C for 8 days—killed with phenyl mercuric nitrate.	.004 c.c.

(5) Agar vaccine—1000 million organisms per c.c.—heat killed .. .005 c.c.
(6) Agar vaccine—1000 million organisms killed with phenyl mercuric nitrate .. .. .005 c.c.

B. *Tests on rats.*

For this purpose, mice weighing between 25 g. and 30 g. were used and white rats weighing 200 g. to 270 g. were used and mouse protective dose was determined.

Vaccine.	Mouse protective dose.	Rat protective dose.
Haffkine vaccine, 4 weeks' growth	.004 c.c.	0.030 c.c.
Broth vaccine—10 days' growth at 37°C.—heat killed.	.004 c.c.	0.020 c.c.
Agar vaccine—1000 million organisms.	.004 c.c.	0.028 c.c.

C. *Toxicity.*

The toxicity of the vaccines tested above was measured as the dose killing 50% of the mice used.

Vaccine.	Toxic dose (dose producing death of 50% of mice used)
Nutrient broth vaccine—28°C heat killed	0.2 c.c.
Nutrient broth vaccine—28°C killed with phenyl mercuric nitrate	0.2 c.c.
Nutrient broth vaccine—37°C.	0.3 c.c.
Agar vaccine	1.0 c.c.
Haffkine vaccine	0.2 c.c.

D. *Titration of the " envelope " antigen content of vaccine.*

For this purpose, quantitative precipitation reaction was employed. Rabbits were immunised with " envelope " antigen prepared from agar growth at 37°C. 0.2 c.c. of serum made up to 1 c.c. was precipitated with 1 c.c. of the supernatant fluid from vaccines under test.

	Mouse protective. dose.	N precipitated.
Haffkine vaccine.	0.004	0.026 mg.
Broth vaccine—10 days' growth at 37°C.	0.004	0.028 mg.
Agar vaccine—37°C.	0.004	0.030 mg.

E. *Detoxicating vaccine.*

Some experiments have been carried out to reduce the toxicity of vaccines by treating them with formalin. Formalin strength of 0.1% seems to be suitable. It reduces the toxicity to less than half and the preliminary experiments show that this amount of formalin does not adversely affect the potency of the vaccine. It is desired to carry out this work to see how these vaccines behave on storage.

24. Plague enquiry in the Salem District, Madras under the Director of Public Health, Madras.

The Hosur Plague Enquiry was continued under the joint finances of the Indian Research Fund Association and the Madras Government. The enquiry comprised of studies of the prevailing rodents and fleas contributing to the endemic plague of the area, intensive application of cyanogas in plague control, and the effectiveness of sulphathiazole in the treatment of plague. X. brasiliensis, X. cheopis, X. astia, Stivalius (sp) and ceratophyllus (sp) are the concerned fleas, the first three predominating on the domestic rodents, and the last three on the field rodents. On R. rattus the brasiliensis index increased from 1.18 in April to 4.6 in October, that of cheopis from 1.04 in April to 3.6 in August, while that of astia did not show any marked fluctuation. Studies of rat nests with respect to brasiliensis and cheopis fleas showed that the former predominated in the ratio of 2.1 to 1 in rat nests on the roofs, while the latter predominated in the ratio of 1.6 to 1 in nests in rat holes.

Over 300 field rats and their fleas were examined for evidence of plague and all were negative. Natural infection was however observed in squirrels on 3 occasions.

In about 15 villages where periodical cyanogas fumigation of rat holes was carried out, plague showed a noticeable reduction from what occurred last year.

Thirteen plague cases were treated with sulphathiazole and eleven recovered.

PHARMACOLOGY.

25. Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore.

During the year the following drugs were investigated :—

Aprajit.

The seeds of *Clitoria ternatea* are used in ndigenous medicines as purgative under the name of Aprajit. The plants were grown in the gardens of the K. E. Medical College from seeds procured from the market and were identified to be *Clitoria ternatea* by the Botanical laboratories of the Punjab University.

The detailed investigation has shown the seeds to contain three glucosides ; two water soluble and the third chloroform soluble. One of the water soluble glucoside has been crystallised and submitted for micro-chemical analysis to the University Chemical Laboratories, Lahore. The melting point of it is 179°–181°C. On hydrolysis it yields an aglucone and sugar which is glucose. The other water soluble fraction could not be crystallised and was obtained as an amorphous powder.

The chloroform soluble fraction, the yield of which is 0.5 per cent, from colour reactions, from solubility and from the nature of the sugar appears to be Cymarine type of glucoside. On purification it has been obtained as a brownish yellow powder which shrinks between 160° to 170° and fuses at 180° to 189°C. It gives colour reactions for sterols and positive Keller Killani test but negative Legal. The sugar that it yields on hydrolysis does not form an osazone.

Both the water soluble and the chloroform soluble glucosides, from colour reactions, seem to belong to the group of cardiac glucosides. The alcoholic extract of the seeds shows feeble toxic action. The powdered seeds given to dogs produce vomiting. Further work on the chemistry and the pharmacology is being carried out.

Brahmi.

Hydrocotyl asciatica. The chemical investigation of this plant is being carried out since 1939. It was reported in 1939 to contain two glucosides and a neutral principle. Samples collected from various places in the Province were examined from time to time till a suitable sample was obtained from a place about ten miles from Amritsar. About 20 seers of the drug were collected and submitted for further examination.

The drug contains oil, resins, a strong reducing agent which resembles vitamin C and a phytesterolin type of glucoside. The glucoside has been crystallized and submitted for microchemical analysis to the University Chemical Laboratories, Lahore. The melting point of it is 250°–252°C. These types of glucosides were described by Power and Salway in 1913 in Jour. Chem. Soc.

The reducing principle was studied according to the method of Szent Gyorgi (1928) but the substance could not be isolated in a pure state to establish its nature. This is believed to be only due to inprocurability and cost of solvents necessary for purification. The reducing principle gives all the colour reactions of vitamin C. The sodium, ammonium, barium and lead salts of this substance were prepared and their solubilities resemble with those of similar salts of ascorbic acid. The reducing principle seems to be associated with a

pigment which makes it resemble a glucoside in reaction. Feeding experiments will be tried to prove its identity with vitamin C.

The resin and the oil have not been investigated.

CHAKSINE.

Pharmacological investigation of Chaksine was continued. Many interesting pharmacological facts have been observed on its effect on heat regulation, its effect in antagonising the effect of acetyl choline and the site of convulsive action has been determined. As Chaksu is used in inflammatory conditions of the eye, the effect of the alkaloid on the growth of staphylococcus has been studied. It was found that staphylococcus exposed to the action of 1 in 100, 00 dilution of the alkaloid for three hours prevented the growth of the micro-organism, whereas dilution of 1 in 400 of it had no effect on B. typhosis or B. Coli.

Following the technique of J. M. Rabson and G. I. Scott (Lancet, Jan. 1943) experimentally produced staphylococcal ulcers in rabbits were subjected to the action of a solution of Chaksine. No decisive result has so far been obtained and the work is still in progress.

Lana-suida fractosa.

The plant yields on successive extraction 19.4 per cent of petroleum ether soluble substances, 2.7 per cent chloroform soluble, 23.5 per cent alcohol soluble and 35 per cent of water soluble extractive.

The analysis of these fractions shows the presence of the following substances :—

Fats, resins and chlorophyll. It also contians tartrates to the extent of 2.5 per cent calculated as potassium bitartrate; carbonyldiurea 1.5 per cent and two crystalline compounds of the purine type. It also contains a small quantity of mallates and traces of citric acid. The ash is 40 per cent. The ash analysis shows the following substances :—

Iron oxide 2% of the ash, calcium oxide 4 per cent and magnesium oxide 0.25 per cent. The rest was a mixture of alkali-chloride and alkaline carbonates. The acid radicles which were present were sulphates, carbonates and chlorides. It also contains vitamin B_1 and C in small quantities. Further studies are in progress.

26. Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay.

PAPAYA SEEDS.

The 'alkaloidal' substance, isolated from papaya seeds and referred to as 'Carpasemine' in last year's report, was further studied, and found to be really benzyl-thiourea. To confirm its identity with benzyl-thiourea, the latter was synthetically prepared and the mixed melting point of the mixture of the natural and synthetic products was determined. There was no lowering of the melting point. The various degradation products obtained from carpasemine have also been identified. Thus the chemical part of the work on carpasemine has been completed and the results have been published in a paper on the subject. The pharmacological work on the substance is in progress and nearing completion.

It is proposed to study the effects of the substance particularly in comparison with those of other thiourea derivatives that have been found active (vide particularly Astwood E. B. Jl. Pharmacol. Exp. Ther., 1943, Vol. 78, p. 79). Preparations of the seeds free from the substance have not been found to have any action.

DAEMIA EXTENSA.

Various extracts of the leaves of Daemia extensa were prepared and toxicity tests on frogs were performed with all the extracts. Attempts were made to find the most active extracts. For example, certain doses of the material extracted with alcohol when injected into frogs killed them within 24 hours. The death of the frogs was preceded by vomiting, sometimes convulsions and always followed by paralysis which was central. In dogs, intravenous injections of the extracts produced a preliminary slight rise of blood pressure with an increase in the spleen volume and a slowing of the respirations. These were the only effects after small doses. But large doses or second and subsequent injections produced irregularities of the heart associated with a fall of blood pressure; the irregularities progressively increased, after larger doses, and the animals died from the arrest of the heart, while the respirations were present. In the earlier stages, the irregularities of the heart could be prevented by atropine or by the section of both the vagi. But after large doses these procedures did not avert the arrest of the heart, while respiration continued. No effect was observed on the movement or tone of intestines of these animals.

Perfusion of the frogs' hearts in situ with different dilutions of the extracts showed no particular or constant effects. Similar effects were observed with the extracts prepared in other ways.

Chemical work with the leaves were carried out with a view to obtain the most active extracts and to isolate the active substance or substances which are believed to be of glucosidal nature.

ERYTHRINA INDICA.

The extracts of the seeds, free from the alkaloid hypophorine, have shown a curare-like action. Attempts were, therefore, made with some success to isolate the alkaloidal substance or substances from the seeds. The chemical work has, however, been interrupted on account of the resignation of the chemist who has joined the University Technology Institute. The work will be resumed when new arrangements will be made for the chemical part of the work.

Some preliminary work has been done on the remaining drugs, particularly on Vinca rosea and Eclipta alba.

27. Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.

RAUWOLFIA SERPENTINA BENTH.

(i) The action of the three alkaloids, *ajmaline*, *serpentine* and *serpentinine* studied earlier failed to explain satisfactorily the use of the drug in cases of insanity. Attempts were therefore made to find out if the plant contains any hypnotic principle and, if so, its probable nature. By eliminating the three known alkaloids viz. *ajmaline*, *serpentine* and *serpentinine* from the total alkaloids of the plant, a sedative and hypnotic principle was detected. The hypnotic

principle antagonises the medullary stimulation of picrotoxin and has depressant action on the medullary centres. The results have been communicated to the Indian Journal of Medical Research.

(ii) A standardised extract of *Rauwolfia serpentina Benth.* was given to patients suffering from various types of mental disorders. All these patients suffered from insomnia, and showed considerable motor and mental agitation. To all these the extract was given twice daily at noon and at bed time in 30 minim doses usually. As the drug has a cumulative and depressant action it is advisable to watch the patients for any manifestation of toxic symptoms. The doses were accordingly controlled. Sleep generally ensued two to three hours after the last dose, and lasted for more than 6 hours. The patients became quiet and behaved more normally and those suffering from effective disorder showed considerable improvement. The alimentary functions of all the patients were stimulated and the appetite improved. A paper on these findings has been published in the I.M.G.

(iii) The total alkaloids obtained from the roots of *Rauwolfia serpentina Benth.* growing in three different regions Dehra Dun, Bihar and Bengal, all cause a fall of blood pressure. According to the extent and duration of the hypotensive effect they may be placed in the following descending order : Dehra Dun, Bihar and Bengal. The Dehra Dun root alkaloid has a depressant action which is purely circulatory. The Bengal and Bihar root alkaloids have in addition, a slight sedative action on the central nervous system, the Bengal alkaloids being more powerful. Respiration is depressed and bronchi dilated by all the three total alkaloids. Uterus is stimulated by all, the Bengal variety being the most active, and the Bihar variety the least potent. All the three alkaloids have dual action on the intestines (a) a preponderating parasympathomimetic action, stimulation of the intestine annulled by atropine and (b) a slight direct depressant action. The isolated guinea-pig intestines react always by relaxation. A paper on these findings has been published in the I.J.M.R.

(iv) A resin has been separated from the extract of *Rauwolfia serpentina Benth.* and washed free from all traces of the alkaloids. It was then separated from the oil contained in it. This oil-free resin was further separated into two fractions an alcohol-soluble and an alcohol-insoluble. Animal experiments proved that marked hypnotic effect was present in the alcohol soluble fraction. This resin fraction is amorphous and tasteless. It causes sedation and hypnosis, the dose varying with the animals. These effects are delayed in manifestation by 3 to 4 hours. The resin acts synergistically with chloralose and ether and the sedative effect prevents picrotoxin convulsion. The site of action seems to be the hypothalamus. The reason of the delayed action is however, not very clear. It may be that a secondary product formed in the body is responsible for the sedative effect. The immediate effect of the resin when given intravenously is slight rise of blood pressure and increased activity of the small muscles of the intestine and uterus. A summary of the results is being sent for publication.

Anthracene purgatives.

Rhubarb.

The hydroxymethyl anthraquinone content of rhubarb obtained from different sources (European, Chinese, Sikkim) has been estimated. The effects of these different rhubarb samples have been tested on cats and it is found that the effect is related to the hydroxymethyl anthraquinone content. Judged by the British or United States Pharmacopoeia standards of purity of rhubarb

the Sikkim samples would have been rejected on two counts—the ash content being too high and the extractive being too low. But pharmacologically these are as good as the European or Chinese rhubarb. It has been shown that samples containing about 2 per cent hydroxy-methylanthraquinone even though they contain alcoholic extractive far below the minimum B. P. standard are pharmacologically as effective as any B. P. standard rhubarb. In view of these findings, a modification of the present pharmacopoeial standard of purity has been suggested. A paper embodying these results has been sent for publication. Further purification of the resin is in progress.

UMBELLATINE.

A new alkaloid called umbellatin has been isolated from Berberis umbellata wall. and Berberis insignis Hook. f. Its pharmacological actions are similar to Berberine but more intense except in producing lesser cardiovascular responses. It has a specific inhibitory action on the growth of Leishmania tropica—the causative organism of oriental sore and a few such cases have been successfully treated with umbellatine. A paper embodying these observations have been communicated for publication in I.J.M.R.

DAEMIA EXTENSA.

It has been found to possess an active glucoside causing a persistent rise of blood pressure and an ergotoxin like contraction of the uterus. The results obtained are encouraging. They are being analysed and will shortly be communicated. A standard method for isolating the active glucoside is being worked out and if the yield is good, it will probably be one of the best ecbolic drugs.

Vitex peduncularis wall var. Roxb. Attempts are being made to isolate the active antihaemolytic principle. Preliminary experiments suggest that it is of glycosidic nature.

Cassia fistula. The root extract of Cassia fistula has been found to possess an active antihaemolytic principle. It is found to be associated with a large quantity of tannin and sugar. The principle appears to be thermostable.

Gurdir. It is a plant from Baluchistan and is supposed to be a good remedy for malaria. It contains traces of alkaloids. The extract of the whole plant is being tried, and the results will be reported in due course.

28. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S. at the Drug Research Laboratory, Jammu, Kashmir.

ACONITUM HERETROPHYLLUM.

The roots of this plant are used in indigenous systems of medicine as antipyretic and antiperiodic.

The active principle of the plant is a non-poisonous alkaloid called *atisine.* It was prepared in the laboratory by following the method of Lawson and Topp's with a little modification. Pharmacologically the principle appears to be inert. When administered intravenously in doses as big as 10 to 15 mgms per kilo body weight, it produces slight fall in blood pressure. Experiments were also put up to investigate the reputed antipyretic property of the principle. It has been found that *atisine* has very slight and uncertain antipyretic effect.

INULA ROYLEANA.

The plant is used for killing lice and for curing boils etc. No systematic chemical analysis of the drug appears to have been done previously. 60 gms. of the finely powdered roots on successive extraction with the solvents yield extracts in the following preparations :—

Petroleum ether 1.79 per cent ; Ether 2.08 per cent ; Chloroform 8.44 per cent ; Ethyl acetate 1.17 per cent ; Alcohol 10.6 per cent.

The petroleum ether extract showed the presence of a pale yellow fixed oil, an essential oil and a little resinous matter.

Ether extract gave further amount of resinous matter and also gave tests for the presence of traces of alkaloids.

The chloroform extract showed the presence of appreciable amount of a bitter substance which gave copious precipitates with alkaloidal reagents.

The ethyl acetate extract too contained traces of alkaloid and resinous matter.

The alcoholic extract did not contain much of the bitter principle. A white crystalline alkaloid has been isolated and further work regarding its purification and pharmacological examination is being continued.

DIGITALIS.

In order to prepare a suitable injectable preparation of 'Digitalis' different fractions of the glucosides of digitalis were prepared in the laboratory from the leaves of Digitalis purpurea. These fractions were then tested for their relative toxicity by the guinea pig method. The conclusions drawn are that the water soluble glucosides have an m. l. d. smaller than the alcohol soluble ones but greater than the total glucosides of Digitalis, i.e. the water soluble fraction is more toxic than the alcohol soluble fraction but less than the total glucosides. Technical detail for the manufacture of the water soluble glucosides is being worked up.

PYRETHRUM.

With a view to find out the most suitable time of harvesting pyrethrum flowers at Kashmir a series of experiments were carried out by picking flowers at different periods of growth of the flower heads viz. unopened buds, half-opened buds (when the apex of the bud just show a yellow tinge on account of the appearance of the flowers), opened flowers (when both ray and disc florets have expanded). In all cases it was found that the maximum yield of pyrethrum was obtained in the open flower stage and from after this there is a gradual decline in the pyrethrum content and a rise in the weight of the flower heads on account of the formation of seeds. So it seems that the most suitable time for harvesting would be to begin it when nearly 50 % of the flowers are open and continue it till the harvest is completed. Such a procedure will of course give a mixed crop of opened and fully opened flowers, but if the interval between the time of open and fully open flower stage is not unduly lengthened, there is very little chance of any decline in the pyrethrum content.

29. Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

The work of the enquiry was carried out on the same lines as in previous years and may be summarized under two heads, viz. (i) Synthetic anti-malarial drugs and (ii) Immunity in monkey malaria.

Synthetic anti-malarial drugs.

A number of drugs of the sulphonamide group prepared by the Chemotherapy Department of the Haffkine Institute were tested for their activity in the rhesus monkey infected with *P. knowlesi*. Out of these, three drugs viz. (a) N' methyl sulphathiazole, (b) 2-N' sulphanilamido-5-ethyl thiazole and (c) 2-N'—sulphanilamido-5-iso-propylthiazole were found to possess a definite antimalarial activity. The Pharmacology of these drugs was, therefore, investigated and their antimalarial activity compared. It was found that drug (a) given orally in a dose of 1.0 gm. per day for two successive days caused a disappearance of parasites but failed to eradicate the infection. In the case of drugs (b) and (c) a similar dose given in the same way was able to eradicate the infection. In the case of drug (b) a single dose of 0.5 g. given only once caused disappearance of parasites from the peripheral blood, but relapse occurred while in the case of drug (c) a single dose of 0.5 g. given only once brought about a radical cure. The question of radical cure was settled by observing (i) the effect of splenectomy, (ii) by injecting the blood of the treated animal into a normal healthy monkey and (iii) by super-infecting the treated animal with a fresh infection. Thus in a treated monkey, showing no parasites in the peripheral blood for more than 3 weeks if splenectomy did not cause a relapse, if its blood was non-infective to a fresh normal animal and if the monkey was readily susceptible to fresh infection, the animal was presumed to be radically cured. The absorption of these drugs was studied by estimating the concentration of the drugs in the blood given in doses which were found to be effective. Drug (a) produced a maximum concentration of 0.89 mg. per cent of the free drug in about five hours and this level was maintained for another three to four hours. Drug (b) produced a maximum concentration of 2.08 mg. per cent of the free drug in about 3 hours, but this level came down to about 1.15 mg. per cent in another three to four hours. In the case of drug (c), the concentration of the free drug was about the same as in drug (b), i.e. about 2.11 mg. per cent in 3 hours and about 1.16 mg. per cent in another three to four hours. The important difference between drugs (b) and (c) however, was that the maximum degree of acetylation was about 60 per cent in the case of (b) and only 17 per cent in the case of (c). Thus, even as low a concentration as about 2.0 mg. per cent of the drug (c) acting over a period of few hours only was able to eradicate the *knowlesi* infection in a rhesus monkey. Drug (c), therefore, has been found to be the most active drug in the limited number of drugs studied.

The drugs varied in toxicity and the drug (a) was found to be the most toxic. The symptom of photophobia was observed in mice treated with this drug (50 mg. per mouse given orally). Drugs (b) and (c) did not show any toxic symptoms in mice in equivalent doses.

Immunity in monkey malaria.

Investigations on the effects of extracts of 'immune' monkeys conferring immunity to normal monkeys were also carried out. The fact that such extracts could confer a certain degree of immunity was observed last year. The present studies were based on a more quantitative basis. It has so far been observed that there is a considerable variation in the results obtained. Some 'immune' spleens showed very little action while others could definitely confer an immunity to normal animals. In most cases where the extracts were found to be active, the parasites appeared in the peripheral blood of the treated animals, increased to a certain extent and then the monkeys went into the stage of chronic infection, without any other treatment whatsoever.

MATERNAL MORTALITY.

30. Enquiry into the causes of infant mortality and morbidity under Dr. Jean Orkney, W.M.S, Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi.

Schedules as attached have been completed for infant deaths during the years 1940, 1941, 1942.

Delhi area—urban area of high infant mortality	302	schedules.
Jungpura semi-rural area of high infant mortality	99	,,
New Delhi urban area of low infant mortality	196	,,
Total	597	

For comparative purposes schedules have been completed for survivors up to 1 year of age put on cards in 1941 and for infants put on cards in 1941 who left the area or were discarded before reaching the age of 1 year.

Delhi area (survivors)	299		
(discards)	105		
Total	404		
		1,001	

The statistical section of the office of the Medical Services has undertaken without charges to punch cards from these schedules and to sort the cards to give the information required.

The schedules have been completed with the assistance of Dr. Pereira, Medical Officer, M. & C. W. New Delhi, and a medical woman paid by the Indian Research Fund Association has therefore not been employed.

1.	Serial number	..	..	1937	38	39	40	41	42				
2.	Year of birth	..	..	1	2	3	4	5	6				
3.	Year of death	..	..	1	2	3	4	5	6				
4.	Name of centre	..	..	1	2	3	4	5	6	7	8		
5.	Month of death	..	..	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.
				1	2	3	4	5	6	7	8	9	10	11	12
6.	Month of birth	..	..	1	2	3	4	5	6	7	8	9	10	11	12
7.	Age of death	..	..	24 hrs.	48 hrs.	1 week	2 weeks	3 weeks.							
				1	2	3	4	5							
				1 month	–2/12	–3/12	–4/12	–6/12	–9/12	–1 year.					
				6	7	8	9	13	11	12					
8.	Sex	..	..	M.	F.										
				1	2										
9.	Housing	..	..	1	2	3	4	5	5+unknown.						
	No. of rooms	..	..	1	2	3	4	5	6	7					
10.	Persons per room	..	..	1	2	3	4	5	6	–8	8+unknown.				
				1	2	3	4	5	6	7	8	9			
11.	Economic status	..	..	–15	–20	–25	–30	–50	–100	–200	–300	–400	–500	500+unknown.	
	Income p.m.	..	..	1	2	3	4	5	6	7	8	9	10	11	12
12.	Income per head	..	..	–2	–3	–5	–7	–10	–15	–20	–40	–50	50+unknown.		
				1	2	3	4	5	6	7	8	9	10	11	
13.	Literacy—Father	..	..	English	Vernacular	Illiterate	Unknown								
				1	2	3	4								
14.	Literacy—Mother	..	..	1	2	3	4								
15.	Purdah	..	..	Strict	Moderate	None	Unknown.								
				1	2	3	4								
16.	Religion	..	..	Moh.	Hindu	Ind. Christ.	Sikh	An. Ind.	Other	Unknown					
				1	2	3	4	5	6	7					
17.	Occupation—Father	..	..	Unemployed	Unskilled	Skilled	Teachers	Clerical	Other professional	Landlords					
				1	2	3	4	5	6	7					
				Employers	Chaprasis	House servants	Unknown	Other	Specify.						
				8	9	10	11	12							

18.	*Occupation—Mother*	*Housework* 1	*Teaching* 2	*Professional* 3	*Domestic occupation* 4								
		House servants 5	Employed outside home 6		Unknown 7	Other 8	Specify.						
19.	Age of mother at cohabitation ..	–12 1	–15 2	–20 3	–25 4	25+ 5	unknown. 6						
20.	Age of expectant mother ..	–15 1	–16 2	–17 3	–18 4	–20 5	–25 6	–30 7	–35 8	–40 9	–45 10	45+ 11	unknown. 12
21.	Pregnancy	Uncomplicated 1	Complicated 2	State complication	Unknown. 3								
22.	Nutrition of expectant mother ..	Good 1	Average 2	Subnormal 3	Poor 4	Unknown. 5							
23.	Health of expectant mother ..	Good 1	Average 2	Subnormal 3	Poor 4	Unknown	Illness-specify. 5						
24.	No. of pregnancies	1 1	2 2	3 3	4 4	5 5	6 6	7 7	8 8	10 9	12 10	14 11	14+ 12
25.	No. of live births	1 1	2 2	3 3	4 4	5 5	6 6	7 7	8 8	10 9	12 10	12+ 11	
26.	„ „ still births	1 1	2 2	3 3	4 4	5 5	6 6	7 7	8 8	10 9	12 10	12+ 11	
27.	„ „ abortions	1 1	2 2	3 3	4 4	5 5	6 6	7 7	8 8	10 9	12 10	12+ 11	
28.	„ „ children living ..	1 1	2 2	3 3	4 4	5 5	6 6	7 7	8 8	10 9	12 10	12+ 11	
29.	„ „ children dead	1 1	2 2	3 3	4 4	5 5	6 6	7 7	8 8	10 9	12 10	12+ 11	
30.	Birth order of present child ..	1 1	2 2	3 3	4 4	5 5	6 6	6 7	8 8	10 9	12 10	12+ 11	
31.	Interval from last birth ..	–9	–12	–15	–18	–21	–24	24+					
	(months) ..	1	2	3	4	5	6	7					
32.	Prenatal care by centre ..	Good 1	Adequate 2	Limited 3	None 4	Unknown 5							
33.	„ „ family	1	2	3	4	5							
34.	Place of confinement	Hosp. 1	Public mat. home 2	Private mat. home 3	Dwelling 4	Unknown 5							

35.	Attendant at home ..	..	Doctor	Centre midwife or dai	Private midwife.								
			1	2	3								
			T. D. private	Untrained dai	Relatives	Unknown							
			4	5	6	7							
36.	Nature of confinement	..	Normal	Abnormal	Specify	Unknown.							
			1	2		3							
37.	Nature of puerperium ..	..	Normal	Abnormal	Specify	Unknown.							
			1	2		3							
38.	Vaccination ..	..	Yes	No									
			1	2									
39.	Condition at birth premature	..	Good	Subnormal	Poor	Malformation	Unknown						
			1	2	3	4	5						
40.	Full time ..	..	1	2	3	4	5						
41.	No. of home visits ..	..	0	1	2	3	4	5	6	8	10	12	12+
			1	2	3	4	5	6	7	8	9	10	11
42.	No. of centre visits ..	..	0	1	2	3	4	6	8	12	16	20	24	24+
			1	2	3	4	5	6	7	8	9	10	11	12
43.	Age at first visit by H. V.	..	–4d.	–1/52	–2/52	–1/12	–2/12	–3/12	–4/12	–6/12	–9/12	–12/12.		
			1	2	3	4	5	6	7	8	9	10		
			Good	Adequate	Limited	Poor	Unknown							
44.	Maternal care Centre ..	..	1	2	3	4	5							
45.	„ „ mother ..	..	1	2	3	4	5							
46.	Family co-operation ..	..	1	2	3	4	5							
47.	Nutrition of nursing mother	..	Good	Normal	Subnormal	Poor	Unknown	Dead.						
			1	2	3	4	5	6						
48.	Health of nursing mother	..	Good	Normal	Subnormal	Poor	Unknown	Specify illness						
			1	2	3	4	5							
49.	Nutrition of infant—6 months	..	Good	Normal	Subnormal	Poor	Unknown							
			1	2	3	4	5							
50.	„ „ „ +6 months	..	1	3	3	4	5							
51.	Health of infant $-\frac{6}{12}$..	..	1	2	3	4	5	Specify illness						
52.	„ „ $+\frac{6}{12}$..	..	1	2	3	4	5							
53.	Birth ..	..	Single	Twins	Multiple									
			1	2	3									

54. *Other influencing conditions* .. *Drink* 1, *Gambling* 2, *Divorce* 3, *Desertion* 4, *Debt.* 5
Domestic unhappiness 6, Multiple 7, Other 8, Specify.

55. Months

	0—3	4—6	7—9	10—12
(a) Breast				
(b) Cows				
(c) Other fresh				
(d) Condensed				
(e) Dried				
(f) Solids				
(g) Unknown				

56. Attendant at last illness .. Hosp. 1, Disp. 2, G.P. 3, Centre 4, Hakim or Vaid 5, Non-skilled. 6

57. History of last illness duration .. 1 day 1, –3 days 2, –1/52 3, –2/52 4, –1/12 5, –3/12 6, –6/12 7, –6/12+ 8

Presenting symptoms—

58. Clinical cause of death.

(a) Stillbirth 1, Birth injury. 2, Premat. 3, Congen. debil. 4, Cong. malf. 5, Specify.

(b) Cong. syph. 1, Jaundice neonat. 2, Umbilical sepsis. 3, Tetanus. 4, Other neonat. sepsis. 5, 6

(c) Digestive 1, Respiratory 2, Infectious 3, Measles 4, Whooping Cough 5, Smallpox 6, Cholera 7, Tuberculosis 8, Other (State) 9, Accidents or injuries 10

58. (*contd.*)

(d) Undefined (presenting symptoms)—
Convulsions 1 Fever 2 Rash 3 Sepsis 4 Jaundice 5 Wasting 6 Spleen + 7 Liver + 8 Anaemia. 9

(e) Inanition— Quality (state deficiency)+
Quality (" ")+

(f) Other causes (specify)

(g) Unknown

59. Investigators remarks.

Cause of death.

Predisposing

Primary.

Secondary

OTHER RESEARCHES.

31. Haematological unit under Dr. C.R. Das Gupta at the School of Tropical Medicine, Calcutta.

Investigation into the problem of blood transfusion.

With the establishment of the Civil and Hospital Transfusion Service at the Haematological Research Department, quite a number of problems bearing on blood-grouping and blood transfusion formed the subject of study during the year.

(a) *Investigation of malaria through transfusion of blood.*

A problem which demands special attention in a malarious country like India is the possibility of transmission of malaria through fresh or stored blood as it did happen in the case which was reported in the Indian Medical Gazette (Vol. 78, No. 8, page 384, 1943). This problem of transmission of malaria through transfusion of stored blood is being studied experimentally in the rhesus monkeys. The effect of cold-storage on the infectivity of the parasitised blood was first studied. For this purpose blood from monkeys infected with *P. knowlesi* was stored in the refrigerator of the Blood Bank at about 4°C. for periods varying from 3 to 7 days. Such chilled and stored blood was later transfused to 11 normal monkeys ; of these 4 were transfused with 3 days' stored blood, 1 with 4 days' stored blood, 1 with 5 days' stored blood, 1 with 6 days' stored blood and 4 with 7 days' stored blood. All these 11 monkeys became infected with *P. knowlesi* at intervals varying from 2 to 16 days. Thus cold storage, by itself, was found quite ineffective in preventing transmission of malaria.

The next point in the programme was to find out how far infection could be controlled with drugs added to the stored blood. Soluseptisine was the first drug to be tried. It was added to 3 samples of parasitised blood in a concentration of 1 in 500 and these were kept in the refrigerator for varying periods and 3 monkeys were transfused as follows—one with 3 days' stored sample, another with 5 days' stored sample, while a third with 7 days' stored sample. As control to these experiments, 2 more monkeys were also inoculated with samples of the same parasitised blood which had been kept in the refrigerator for 3 and 7 days, respectively, but to which no soluseptisine had previously been added. All the monkeys became infected with *P. knowlesi*, in due course, regardless of the fact whether soluseptisine had previously been added or not to the sample. Further work on this line is proceeding.

(b) *Regeneration of blood after blood donation.*

To study the regeneration of blood in donors, full blood counts were done in each individual, once, just before donation of blood and later, once daily, after donation of blood for 7 to 10 days, according to the time taken by an individual to reach his pre-donation blood level. So far 8 donors were examined ; out of these only 4 donors presented themselves fairly regularly, according to schedule. As for the remaining 4 donors, one showed no anaemia, having donated only 120 c.c. of blood, while the other 3 did not bother to come more than once or twice for blood examination. This work will be continued further and it is hoped that more and more of such donors as will submit themselves for repeated blood examinations will become available.

(c) *Rh. factor.*

In recent times, the Rh. factor of blood, has often been incriminated as one of the aetiological factors in many, hitherto unexplained haemolytic reactions, following certain homologous-group blood transfusion. The preliminary experiments to prepare anti-Rh agglutinin by immunizing rabbits with blood of rhesus monkeys have given very encouraging results. The process is being further developed to study the clinical significance of the Rh factor in homologous-group blood transfusions.

Work in the blood bank.

As during last year, so this year also, owing to the great rush of work in the Blood Bank, the services of Dr. D. N. Majumder was lent temporarily for a month there. Work, here, consisted mainly in collecting blood from donors who had been found fit for such donation on previous medical examination.

Normal haematological standard for the agricultural labourers of Bengal.

Lack of any representative data for retarding the normal blood values of the agricultural labourers of Bengal led to the investigation of their blood picture in a village in the vicinity of Calcutta. The original intention was to examine a fairly large number but circumstances did not permit to examine more than 29 individuals—all apparently healthy and working on the fields and all belonging to the same economic status. Of these, one was discarded and the data for the remaining 28 are shown in table I.

Van den Bergh reaction in all these cases was negative. In 12 of these cases where the stools were examined for hookworm ova, 3 cases did not show any infection, in 8 cases there was slight infection and only in one was the infection moderately high. Eosinophil counts were moderately high in most of these cases. Compared with the findings on a similar series of 24 normal tea-garden coolies, (Napier and Das Gupta), it is obvious that notwithstanding their similarity in standards of living, the agricultural labourers of Bengal differ markedly in blood picture from their compatriots in Assam with their lower haemoglobin level and smaller size of red cells.

Blood picture of the normal monkeys.

In connection with the various animal experiments, performed in this laboratory from time to time, a large number of normal blood counts were done on monkeys. These counts were analysed this year with a view to obtaining the mean and the standard durations of a series of 243 counts from 59 rhesus monkeys. The results are reproduced in table II.

Further experimental work on animals.

This work was continued on much the same line as in the previous year.

(a) In order to accumulate further data on the effect of repeated small blood withdrawals on the haemopoetic system of monkeys, 2 more monkeys were bled regularly from day to day. In the first one, bled 9 c.c. a day for 56 days, haemoglobin fell from 14.58 gm to 3.57 gm and red cells from 6.23 millions to 2.07 millions, while in the second, bled 5 c.c. a day for 27 days, the corresponding drop was from 14.16 gm to 6.19 gm and from 5.46 to 3.47 millions, respectively.

TABLE I.

Haematological standard for the agricultural labourers of Bengal.

Haemoglobin in gms per 100 c.c. of blood.		Red cell in millions per cu mm.		Reticulocyte percentage.		Packed cell volume in haematocrit		Mean cell voleum in μ^3.		Mean corpuscular haemoglobin in YY		Mean corpuscular haemoglobin concentration percentage.		White cells per cu mm.	
M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.
15.12	± 1.90	5.44	± 0.25	0.3	± .03	54.4	± 6.9	32.2	± 6.9	27.2	± 5.4	33.7	± 5.6	8,900	± 1,400

TABLE II

Haematological standard for the Tea Garden Coolies in Assam.

Haemoglobin in gms per 100 c.c. of blood.		Red blood cell in millions per cu mm.		Reticulocyte in per cent of red cells.		Packed cell volume.		Mean cell volume in μ^3		Mean corpuscular haemoglobin in YY.		Mean corpuscular haemoglobin percentage.		White cells per cu mm. of blood	
M.	S.D.	M.	S.D.	M.	S.D.	M.	S.D.	M	S.D.	M.	S.D.	M.	S.D.	M.	S.D.
13.59	± 1.04	5.76	± 0.81	1.19	± 0.8	44.7	± 2.7	78.5	± 2.5	23.8	± 2.5	30.4	± 1.9	15,340	± 4,300

M—Mean.
S—Standard deviation.

In confirmation of the previous findings, it was observed that in the downward progress of anaemia, the tendency towards hypochromicity was a more marked and a more constant feature than that of microcytosis. Further, the experiments also confirm the previous finding that there was a marked individual variation in the natural power of blood regeneration ; for while the more anaemic first animal took only 43 days to regain its normal blood level, it was not till 76 days that the blood values of the less anaemic second animal reached the normal levels.

(b) Last year's experiments on the combined effect of the deficient diet and M & B 693 on the haemopoetic system of 4 monkeys were concluded in the early part of the current year, when 2 additional monkeys were taken on the book. Of these 6 animals, one died during the course of experiment, a second developed lung complications and began to refuse the test diet in the middle of the experiment, while a third, though quite healthy, could not be induced to take the artificial diet, presumably due to its intense dislike for cod-liver oil and olive oil which formed integral parts of the diet. The remaining 3 which received a daily dose of 0.25 gm/kg, 0.30 gm/kg and 0.33 gm/kg for 14.7 and 12 days, respectively, did not behave uniformly ; for the first and the last often went off their food for days together, while the second consumed the test diet indifferently. It was pretty obvious that the artificial diet, made up of rice, salt-mixture, cod-liver oil and olive oil to give it a high fat and low protein content was such that these animals very soon acquired a positive hatred for it. Hence, the results obtained under such experimental conditions cannot with fairness be compared with those secured by administering M & B 693 to monkeys, kept on normal stock diet, which they consumed with remarkable avidity.

As in previous years, Dr. Majumder always assisted in the routine work of the department.

32. Enquiry into medical mycology under Dr. L.M. Ghosh at the School of Tropical Medicine, Calcutta.

In this year the work was confined mostly to the studies of the ringworm and the preventive measures applicable.

Studies of ringworm.

During this period 310 cases of ringworm of the body, nails and hair were investigated as follows :—

Ringworm of the body and nails—Total number of cases were 212 of which 200 cases were Epidermophytosis and 12 were Trichophytosis.

Ringworm of the hair—Total number of cases were 63 of which 21 cases were Sycosis barbae and 42 were pustular folliculitis.

Ringworm of the scalp—Total number of cases were 11 of which 6 were Microsporosis and 5 were Trichophytosis.

Dermatomoniliasis—Total number of cases were 24.

Epidermophytosis.—This is the most common fungus infection in India causing ringworm of the body and nails and affects all races and all classes of people. The fungus could be demonstrated by microscopic examination in 94 per cent of cases of primary infection. Culture was positive in 95 per cent and

showed that of the 212 cases of ringworm of the body, 194 cases were due to *Epidermophyton floccosum*, 6 cases due to *Epidermophyton rubeum* and 12 cases due to Trichophytosis.

Trichophytosis :—

A. *Trichophytosis of the skin* is not common in India and not usually recognised. The fungus can be differentiated by culture only. Its incidence in India has not been stressed. Both microscopic and cultural examinations were positive in 95 per cent of the cases.

Ringworm of the hair.—

B. *Sycosis barbae (tinea)*—21 cases in this series. Only 20 per cent gave positive results in culture. *Tr. violacium* was the fungus usually found.

C. *Pustular folliculitis (tinea)*.—Common amongst the labourers. The primary site is usually the legs from which the infection spreads to the other parts of the body. Fungus cannot be easily demonstrated. Less than ten per cent of the cases gave positive results in culture and *Tr. violacium* was usually found.

Ringworm of the scalp.—

(1) *Trichophytosis of the scalp*—All the 5 cases in this series gave positive results in microscopic and cultural examinations. *Tr. violacium* being the commonest fungus causing ringworm of the scalp in the *Indian children.*

(2) *Microsporosis.*—All the 6 cases gave positive results in microscopic and cultural examinations. *M. audouini* is the more common Microsporosis causing ringworm of the scalp in the *children of non-Indian origin.* (A paper has been sent to the press for publication about the *M. audouini* infection in India).

Dermatomoniliasis—Monilia infection of the skin and mucous membrane simulating ringworm is not uncommon in India—being 24 cases in the series. It affects (a) the hands and feet between the fingers and the toes (intertrigo) ; (b) the nails—causing onychia and perionychia; (c) the mucous membranes of the mouth and the external genitalia. Microscopic examination for the fungus was not satisfactory but culture gave positive results in 80 per cent cases.

It appears from the above that in India *Epidermophyton floccosum* is the most common fungus causing ringworm of the body and *Trichophyton violacium* is the common fungus causing ringworm of the hair.

Preventive measures.

The work on the preventive measures was done against ringworm of the body and consideration was given to the following points.

The drug or drugs tested should be cheap, easily available in India, non-irritant and non-corrosive, non-staining to the clothes, and as could be easily administered.

Tartaric acid, acetic acid and phenol were first tried and their fungicidal properties were determined. It seems that acetic acid and phenol have some effect in preventing the infection when used in the strength of one per cent in watery solution or better still as one per cent in 20 per cent alcohol. Tartaric acid is not satisfactory. Other drugs are also being tried for better results.

NAGA SORE OR ULCUS TROPICUM.

The occurrence of this disease has never been reported before from Calcutta and its suburbs and even from any other part of West Bengal. During the last monsoon months there was a sudden epidemic of this sore in Calcutta and the suburbs. The victims were mostly the destitute evacuees who poured into Calcutta from the villages. The incidence was enquired into and experiments were done to find out the methods suitable for mass treatment of these sores in the camps. A paper has been accepted by the press for publication.

33. Studies in tests of physical fitness in Indians under Dr. D.M. Telang at the Grant Medical College, Bombay.

The object of the enquiry is to see how far the various tests said to grade the 'physical fitness' of an individual as used in America and England are applicable to Indians and to work out the standards for Indians in these respects if possible. No such data have so far been available in this country.

As has been reported before observations on the following tests and measurements have been done on each of more than 600 subjects mostly in the age group 18 to 29 and comprising both males and females, normal and diseased. Some of the results obtained were given in the last summary report.

I. Circulatory tests—(i) the Schneider test of cardiovascular rating, (ii) the Crampton 'blood ptosis' test, and (iii) McCloy's test.

II. Respiratory tests—(i) breathholding test, (ii) expiratory force, (iii) persistence or fatigue test, (iv) vital capacity, and (v) Flack-Woolham index of fitness.

III. Body measurements—(i) height—standing and sitting, (ii) weight (iii) surface area, (iv) chest—resting, inflated and deflated, (v) trunk area, (vi) abdomen, and (vii) nutritional indices— V. Pirquet's 'pelidisi', Rohrer's, and Davenport's 'index of build'.

SCHNEIDER TEST.

After dividing the subjects into several groups and sub-groups a statistical study on the Schneider test was undertaken.

By repeating the test on 27 individuals its 'reliability coefficient' was found to be 0.81. The test may hence be considered sufficiently accurate for its purpose.

The influence of the following factors on the test were then studied.

Influence of exercise.—If it be assumed with the world opinion that exercise improves the 'physical fitness' of a person then this test ought to differentiate persons taking exercise from those that are not by giving better results in the former. Actually this was found to be the case. Normal male subjects divided into three groups (i) 'No exercise' (2) 'Moderate exercise' and (3) 'Athletes' could be 'significantly' separated from one another statistically by their 'mean' scores.

In order to eliminate the effect of the body size the scores were reduced to units per sq. m. of body surface. Even then the groups could still be differentiated.

Similar results were obtained after comparing the scores of persons grouped after keeping the diet, the time of day, and the diet as well as the time of day constant.

The test may therefore be said to grade the 'physical fitness' fairly well assuming that regular exercise improves the 'fitness'.

Influence of sex :—The corresponding male and female normal 'No exercise' and 'Moderate exercise' groups could be 'significantly' differentiated suggesting a sex influence on the test. This was so even after keeping the diet or the time of day constant.

When however the scores were reduced to units per sq. m. of body surface the 'significant' difference between the corresponding groups was seen to disappear, even on keeping the diet or the time of day constant.

This possibly suggests that sex does not influence the test so long as the body surface is kept constant.

Influence of diet.—Subgroups belonging to the same exercise group but having different diets (vegetarian and non-vegetarian) could be differentiated 'significantly'. This was so even on keeping the time of day and the body surface constant.

This possibly means that the diet has no influence on the test.

Influence of community.—Subgroups Guzerathis, Deccanis, Parsis, Muslims etc. belonging to the same exercise group could not be differentiated even on keeping the body surface constant.

This suggests that the community influence on the test is nil.

Influence of interval between the test and the previous meal.—As the test involves observations on the pulse rate and the blood pressure the interval between the test time and the previous meal may, it was thought, affect results. It was noted that the scores of persons in the same exercise group but whose interval was 2½ hours were not significantly different from those of others who had intervals of more than 2½ hours.

Influence of time of day.—This was studied in a group of 23 subjects on whom the test was done in the morning and in the afternoon separately keeping the same interval between the test time and the meal time. They did not give 'significantly' different results even on keeping the body surface constant.

Hence the time of day probably does not affect the test.

Similar studies on the other tests have yet to be done before determining which of them is the best for grading the 'physical fitness' of an individual.

34. Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta

It may be pointed out that in spite of the difficulties arising out of the present emergent situation, the enquiry has been able to continue the main lines of work conducted during the previous years, and, in addition, to undertake work on fresh lines. Considerable knowledge regarding the various aspects of the disease in this country has been gained during the year. The work carried out during the year 1943 may be dealt with under the following heads :

Field work.

During this year, field surveys in the districts of Nasik in the Bombay Presidency and in Muzaffarpur in Bihar were completed. Nasik has an altitude of 2,000 feet and a dry climate ; *Culex fatigans* is common but there is no

indigenous filarial infection. The infection observed in Muzaffarpur was entirely due to *W. bancrofti* : *Culex fatigans* were present in large numbers but no species of *mansonioides* were present.

Morphology of filaria from different areas in India.

Further material of blood smears from residence of Damda village (Drug, C. P.) was examined and it was found that the infection here was entirely *Wuchereria malayi*. No bancrofti infections were observed. This area has been found to have a fairly high incidence of filariasis (22%) but the manifestations are entirely elephantiasis of legs and hands. No cases of genital affections or of chyluria were recorded, a characteristic of areas with *W. malayi* infection.

From a dilated lymphatic in the arm of a case of chyluria from Biharsharif (Bihar) an adult filaria was aspirated and later identified as *W. bancrofti*. The fluid aspirated from the lymphatic as well as the peripheral blood collected at night were positive for microfilariae of *W. bancrofti*.

Studies were made of microfilariae of *W. bancrofti* obtained from lymphatics, hydrocele fluids and peripheral blood of infected persons freshly arrived in Calcutta from North Bengal (Dinajpur), West Bengal (Midnapur), Orissa (Cuttack) and U. P. (Benares). There were no differences in the appearance or measurements of microfilariae from the different sources.

Filarial infection in animals.

Filarial infection in a pariah dog in Calcutta was examined and identified as *Dirofilaria repens*. This infected dog was kept under observation to determine if the microfilariae showed any periodicity. It was observed that the microfilariae of *D. repens* did not show any increase in number in peripheral blood at night as compared with the number in blood collected during daytime.

Filarial infection of horses has been found to be fairly common in Bengal. The microfilariae measure about 120 μ long, 3.5 μ broad and devoid of sheath. The specific determination of this microfilaria has not yet been made. It is not known whether the infection has any pathological effect on the host.

Another species of microfilaria which is sheathless measures 190 μ long, and is considerably thicker than the previous form. It has been collected from the blood of horses in Assam and has not yet been identified.

Studies on the periodicity of microfilariae.

To determine the life and mechanism of periodicity of microfilariae transfusion experiments were started last year, and were continued during the present year. Hydrocele fluid with microfilariae was injected into hydroceles free from microfilariae. Out of four such transfusions carried out, two cases showed microfilariae for varying periods. In one case, an acute attack of lymphangitis of the scrotum followed the transfusion and filarial embryos were observed on the third and fourth days after which time they disappeared ; while, in the second patient they were present for two weeks after which period the hydrocele turned into a haematocele and no embryo could be detected in the fluid. In the remaining two cases the fluids injected together with the original hydrocele fluid were completely absorbed in 3 to 4 days after the transfusion. The results are very interesting and the work is being continued.

Analysis of hydrocele fluids.

129 patients with hydroceles were examined during the year. Hydrocele fluid was aspirated in 110 cases. The fluids were serous and clear in 96, mixed

with blood in 6, chylous in 4, and with excess of cholestrin crystals in 4. Microfilariae were present in three of the chylous and in four of the clear fluids.

Filarial allergen.

The successful treatment of allergy in filarial patients by the administration of filarial allergen and the value of filarial allergen in the treatment of filariasis has been reported previously. In view of the instability of filarial allergen, the globulin fraction has been isolated and the determination of its value in the treatment of filariasis is in progress.

Complement-fixation test.

Complement fixation tests have now been devised for various bacterial, protozoal and metazoal diseases, and for the identification and differentiation of such substances as serum, blood, milk, etc. In view of the interesting results obtained with the W. K. K. antigen recently in kala-azar, it was tried in filarial cases also. The results, however, turned out to be very poor as in the case of tuberculosis. Several experiments were also carried out to see if hydrocele fluid and alcoholic extract of *Dracunculus medinensis* would be useful as antigens for complement-fixation test in detecting filarial infection. The experiments gave no positive results. This work is being continued with antigens prepared from other helminths and acid-fast bacilli.

Filarial lymphangitis.

Blood was cultured for bacteria in two cases of filarial lymphangitis during acute stages. The cultures did not show any growth in both cases. In one of the patients, the blister fluid was also cultured for secondary infection, but the result was negative.

W. B. C. count during the acute stage of lymphangitis showed a moderate leucocytosis—14,000 to 20,000 per c.mm. The differential count showed an increase from 80 to 90 per cent of the polymorphonuclear cell.

Past histories of filarial lymphangitis in patients with filarial diseases were recorded. A series of 250 case records were completed. So far as could be judged with the limited data, the incidence of infection in the males is greater than in the females.

Amputation. Amputation of the elephantoid limb is recommended when the limb has become riddled with abcess cavities and sinuses or where gangrene threatens with consequent danger to life. More frequently, an amputation is carried out to remove the disability due to the heaviness of the elephantoid limb and there is a feeling that it is a measure of treatment of filariasis. The history of a young and robust man who had a moderate elephantiasis of the leg which was amputated in 1923 was therefore carefully followed and the results were published during the year. Onset of elephantiasis of the stump of the leg and the recurrence of lymphangitis showed that the filariasis was not cured by amputation, but of course the patient was relieved of the weight of the elephantoid tissue of the leg.

Other laboratory work.

Work on the estimation of bacteriolytic power of sera of filarial patients both before and after treatment with mixed filarial vaccine is in progress. In a small number of cases observed so far, the vaccine treatment appears to enhance this property in the sera.

Estimation of the pH of clear hydrocele fluids was made in 25 specimens and it was found to range from 7.4 to 7.8.

A similar estimation of the sweat collected from ten filarial cases was made and it was found to range from 4.5 to 6.

W. R. test of hydrocele fluid and of blood of the same patient were made in 12 cases. The hydrocele fluid gave a positive reaction where the blood was positive.

TREATMENT OF FILARIAL INFECTION.

The results of the trials carried out with various metallic and synthetic compounds, and vegetable drugs in the treatment of filarial infection have been reported from time to time. During the current year the following preparations were tried :

Acetylarsen—a pentavalent compound of arsenic injected intramuscularly. (May & Baker, Ltd., London).

Preparation of mercury—Ung. Hydrarg & Hydrarg oleatus applied locally.

'M & B 800'—A stilbene compound synthesised by Messrs. May & Baker, Ltd., London, given intravenously.

Gentian violet solution injected intravenously.

'M & B 693', 'M & B 760' and sulfadiazine—compounds of sulfanilamide administered orally.

Pedunculine—injectable extract of Vitex peduncularis.

Hydrocele asiatica—extracts of fresh green leaves administered orally.

Embelia seed powder—given by mouth in one dose of 2 drs. on empty stomach followed by saline purgative after 2 hours.

Opium—administered orally and as observed in opium addicts.

As a specific for filarial infection, none of the above was found to be of any value.

35. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras.

VACCINIA VIRUS.

(A) Field experiments to ascertain the relative value of chick membrane lymph, and the routine calf lymph in the prevention of small pox were continued. A detailed report on the findings in this, the third year of the experiment, has already been submitted to the Public Health Commissioner with the Government of India. Some essential features are noted below :—

In our previous experience with the cultivation of membrane virus serially, an occasional drop in the potency was noted in some passages, which was made up in the subsequent ones. There was no deterioration of potency of membrane virus after serial passage in eggs extending over three years.

An analysis of the field results for the third year of the experiment gives a case success rate of 86.1% and insertion success rate of 69.1% in 720 cases of primary vaccination done with membrane lymph as compared with 99.1% and 97.3% in 774 cases of primary vaccination done with calf lymph.

In 845 revaccinations with membrane lymph, the case and insertion success rates were 12.5% and 7.8% as compared with 43.6% and 31.8% respectively in 883 revaccinations with calf lymph.

A difference in the case success rates with membrane lymph in the two age groups, viz., under one year group, and 1 to 5 years group, was noted in the first two years. It was first suggested that this difference might be due to the possibility of the Tyrode Membrane Lymph being wiped out more readily as it was less viscous than calf lymph. The same difference in the case success rates in the two age groups has been noted this year also even when glycerinated membrane lymph has been issued. Since this variation has been obtained with Glycerine Tyrode Lymph in a dilution of 1 in 5 also, the question of the consistency of lymph having any bearing on these reults does not arise.

It might be that other factors influence this result.

There might be some residual " maternal " immunity present in children under 1 year of age, which progressively disappears. A " weak " lymph may not be able to get over it.

Calf lymph, however, is not a pure suspension of Vaccinia Virus as membrane lymph is, but contains in addition a few bacteria and bacterial products. These add to the " invasive property " of lymph as investigated by Duran-Reynals. By the addition of bacterial extracts, testicular extracts etc., Duran-Reynal has shown that a good and vigorous " take " can be obtained from an initially weak lymph. Membrane lymph on the other hand, does not contain this Duran-Reynal Factor in any appreciable amount as it is free from bacteria and bacterial products. To that extent, membrane lymph might be considered as " weak lymph ". That this may really be the case is further corroborated by the very low case success rate obtained in revaccination cases with membrane lymph. For, in these cases, different grades of immunity are encountered which that lymph has to overcome.

(B) Nagler had reported that the phenomenon described by Hirst of agglutination of chicken red cells by Influenza Virus and inhibition of the agglutination by specific immune serum was found to be applicable to Vaccinia Virus and Vaccinial immune serum. This observation, if confirmed, would be of importance, for, not only would it provide a suitable technique for the " quantitative " determination of Vaccinial antibodies in human sera, but would also throw doubt on the validity of the results obtained in influenzal antibody titrations in subjects previously vaccinated with vaccine lymph. Attempts to confirm the above findings, using both membrane and calf lymphs, have been unsuccessful. The work is, however, being continued.

Typhus.

Attempts to establish two strains of typhus—the Breinl and Madrid strains —received from America in a dried form, both by serial guineapig passages and serial yolk-sac cultivation were made. The results were negative, and it was concluded that the strains as received were not viable.

Infective hepatitis.

Studies initiated last year were continued.

In a small epidemic of jaundice in an institution, five out of nine cases were taken for investigation. From three cases, serially passageable lesions were obtained on chorio-allantoic membrane, both from throat washings and from sera obtained after the onset of jaundice, but during the afebrile period. After

the 8th serial passages in most cases, the virus, if any, had died out. Attempts to infect monkeys (*M. sinicus*) by intracerebral, intravenous and subcutaneous routes, using infective serum, proved unsuccessful.

Pooled serum from four cases of jaundice in another epidemic did not produce any lesion on the chorio-allantoic membrane of the developing egg.

Two sera were inoculated into eggs by the allantoic route and the liver and the allantoic fluid tested for the presence of the infective agent. The results were inconclusive as the sera too did not produce any lesions on the chorio-allantoic membrane in the first instance.

LYMPHOGRANULOMA.

In August 1943, studies on the cultivation of Lymphogranuloma inguinale Virus in eggs were undertaken for the preparation of Yolk-sac antigen "LYGRANUM".

36. Kala-azar enquiry under the Director, Pasteur Institute, Shillong.

During the year, the enquiry attempted the solution of the following problems—(1) the number of infected sandflies necessary to bring about infection in white mice ; (2) to determine any difference in the intensity of infection or in developmental forms in two batches of laboratory bred sandflies fed on a kala-azar case and thereafter maintained on (a) raisins and (b) blood feeds, respectively ; (3) continuing the studies on the bionomics of the sandfly.

The results of the findings are set out below.

THE NUMBER OF INFECTED FLIES NECESSARY TO BRING ABOUT INFECTION IN WHITE MICE.

A series of fourteen mice were used, but as four animals died within a fortnight from other causes they are excluded from the results. Thus ten mice received bites from infected flies, which were proved to be heavily infected on subsequent dissection. The bites ranged from one to five. The experimental animals were under observation for periods ranging from 47 to 243 days. In none of the animals could infection be detected from an examination of smears of internal organs. These experiments show that the white mouse is not very susceptible to this infection and that heavier doses may have to be employed to bring about infection.

EXPERIMENTS PERFORMED TO DETERMINE IF DIFFERENCES EXIST IN THE INTENSITY OF INFECTION OR DEVELOPMENTAL FORMS IN FLIES MAINTAINED ON BLOOD AND RAISINS, RESPECTIVELY, AFTER THE ORIGINAL INFECTING FEED.

After a feed on a kala-azar case flies were divided into two batches, one maintained on raisins and the other on blood feeds on mice. Flies fed on sixteen kala-azar cases were observed, but in five there was no development of flagellates in either batch, but those that fed on the remaining eleven cases showed development of flagellates. The attached table gives the results.

On analysing the results, it is seen that there is little difference in the two batches in regard to rate of infection ; it is 23.1% in raisin-fed and 22.5% in blood-fed flies, the number of flies employed being 199 and 243, respectively. The intensity of infection is more or less the same in the two batches, the great majority

Raisin fed.						Case No.	Mouse fed.					
No. fed. No. +	7th day.	8th day.	9th day.	10th day.	11th day.		6th day.	7th day.	8th day.	9th day.	10th day.	No. fed. No. +
$\frac{25}{10}$					5++++ 5++++	123	1+++ 1++	1+++	1++++ 2+++ 1++			$\frac{33}{7}$
$\frac{33}{4}$				1++++ 2+++ 1+++		124	4+++	1++++ 3+++	5++++(T) 2+++	2+++ (T)[2] ++++ (T) 2+++++		$\frac{91}{21}$
$\frac{22}{7}$				4++++ 2+++ 1++		125		2+++	1+++	1++++ (T) 1+++		$\frac{24}{5}$
$\frac{24}{1}$				1+++		130		1++++ 1+++ 1+	1+++	2+++ (T)		$\frac{24}{6}$
$\frac{14}{1}$				1+++		131			1++			$\frac{14}{1}$
$\frac{9}{6}$	1++++ 3+++	2+++				132			1++++ 1+++ 2++			$\frac{9}{4}$
$\frac{11}{5}$			1+++	2++++ 1+++ 1++		133				1++ (T)		$\frac{13}{1}$
$\frac{28}{3}$		1++++ 2+++				135		2++	1+++			$\frac{9}{3}$
$\frac{11}{4}$				1++++ 3+++		137			1++			$\frac{11}{1}$
$\frac{18}{3}$	1+++	1+++ 1++				143		2+++ 1++	2+++			$\frac{13}{5}$
$\frac{4}{2}$		2++				146		1++				$\frac{3}{1}$
$\frac{199}{46}$	Total.			Note — (T) stands for twice fed.							Total.	$\frac{245}{5}$

showing heavy infection ; still, a greater percentage of intense infections (++++ and +++) were to be seen in the raisin-maintained group. Another finding which may have its significance is that whereas in the blood-maintained flies 80% of infected flies had died before the ninth day and only 20% survived on the ninth and tenth days, in the case of raisin-maintained flies, this ratio was reversed, 70% surviving from the ninth to the eleventh day and 30% from the seventh to eighth day. Apart from other factors that might be involved, the possibility that the longer sojourn of flagellates in the fly might endow them with that degree of virulence as to bring about infection in the human being, suggests itself.

BIONOMICS OF SANDFLIES.

(a) Laboratory bred sandflies when kept on raisins alone lived for a maximum period of 12 days as compared to flies maintained on blood which lived for 19 days. During this period the fly took five blood meals and laid a batch of eggs three to four days after each blood meal. Flies fed on raisins did not show any development of ova and did not lay eggs.

(b) Longevity of flies fed on blood first and subsequently maintained on raisins :—Flies were fed on a warm-blooded animal and thereafter maintained on raisins. After the first oviposition which took place on the 4th or 5th day after the blood meal, no more eggs were laid by the flies. An analysis of 473 flies shows that only 40 or less than 10% were alive after the tenth day. The impression gained is that there is a seasonal difference ; flies living longer in certain seasons than in others. This however requires confirmation.

(c) Comparison of feeds on different mammals : Flies were fed first on a warm-blooded animal as man, calf, mouse, monkey and were kept individually in tubes. No more feeds were given. The average duration of life was found to be for man 4.8, calf 4.6, mouse 4.9 and monkey 4.1, thus showing little difference.

(d) Longevity of flies fed on raisins first and subsequently on blood : For the first five days after emergence, flies were maintained on raisins and then were fed on man. The duration of life was not appreciably lengthened as seen from a series of 52 flies. Of these only 31 flies were alive on the fifth day for the blood feed. The average length of life of these was 8.6 days.

(f) Determination of the pH of the gut of *P. argentipes :* The pH determinations were carried out in respect of the midgut and hind gut and the anterior and posterior portions of the midgut, as it was hoped they might throw a light on the tendency of flagellates in infected flies to aggregate towards the anterior end of the midgut. Both uninfected and infected flies were employed. The B.D.H. capillator was used for estimating the pH. No significant differences were observed. The readings in all were in the region of 7.2 to 7.4.

(g) Determination of the blood meal of wild sandflies by precipitating sera : The object was to determine the natural preference of flies as between man and cattle, where opportunities for such feeds were about equal. Fed flies from selected localities have been caught and filter paper preparations made, but for want of potent sera, the work could not be completed.

IV. MISCELLANEOUS.

(1) 41,371 sandflies were bred out in the laboratory (21,163 females) during the year. 9,146 flies were caught for breeding purposes. During this year the month of November was the best as regards prevalence of flies. Last year it was October.

(2) Three cases of post kala-azar dermal leishmaniasis were discovered in the same family. On one of them in whom parasites were seen microscopically in skin clippings, 24 *P. argentipes* were fed, but no development of flagellates occurred in any of the flies.

(3) Eighty-eight patients suspected to be suffering from kala-azar were examined by the aldehyde test as well as by microscopical examination of peripheral blood. Forty-five of them were proved to be suffering from kala-azar ; in thirty-three *Leishmania donovani* were demonstrated in peripheral blood. Sixteen cases proved to be suffering from malaria and the remaining cases were negative to all examinations.

(4) During the course of searches for kala-azar cases in villages blood smears from suspected persons were microscopically examined. *L. donovani* were seen in three cases and malaria parasites in seven.

(5) Sera of kala-azar cases have been collected for immunological studies.

37. Enquiry on anaerobic wound infections under Dr. R.G. Dhayagude at the Seth G.S. Medical College, Bombay.

Isolation of anaerobic organisms.

During the course of the last eleven months, the work of isolation of anaerobic organisms, from various sources has been continued. Material from 49 new cases was added making up a total of 208 cases studied so far. The distribution of these new cases was as follows :—

Surgical	.. ..	30
Post-mortem	.. ..	10
Samples of human stools	.. ..	5
Sample of guineapig stool	.. ..	1
Other sources	.. ..	3
	Total ..	49

Strains of anaerobic organisms.

17 new strains of anaerobic organisms were isolated from these cases. They have been identified after studying their morphological, cultural and biochemical characters. Animal pathogenicity of these organisms was tested by using white mouse as a test animal. The incidence of these new strains was as follows :—

Clostridium	welchii	.. ..	5
,,	septique	.. ..	2
,,	tetani	.. ..	2
,,	sporogenes	.. ..	8
		Total ..	17

Aerobic cultivation of anaerobes.

Two sets of media, one containing digest broth with addition of sodium thioglycollate, glucose and methylene blue in amounts recommended by Brewer

and the other containing plain nutrient broth to which a sterile nail was added, were prepared. Old cultures of Cl. welchii, Cl. tetani. Cl. histolyticum were inoculated in these media and growth was noted after the aerobic incubation of 24, 48, 72 hours. It was noted that both the media supported the growth of Cl. welchii satisfactorily while they were unable to do so with Cl. tetani and Cl. histolyticum which require more rigorous conditions of anaerobiosis.

TOXIGENIC CAPACITY OF CL. WELCHII.

Four recently isolated strains of Cl. welchii were tested for their toxigenic capacity using the usual medium and procedure. Cl. welchii S. R. 12 the standard toxigenic strain was used as a control. It was noted that none of these produced any toxin (which was lethal to mice) while the control stain yielded toxin 0.2 cc. which killed the mice injected intravenously within 20 hours. All these strains were lethal to mice when injected intramuscularly.

Similarly an old strain of Cl. welchii No. 4 was taken up to study its toxigenic capacity. It was passed through white mice successively for 8 times in order to raise its pathogenicity to its original level and then it was tested for toxin production in the usual medium. Even after regaining its original pathogenic capacity it failed to yield any toxin.

As it was found out that most of the strains of Cl. welchii isolated since the beginning of the enquiry failed to yield toxin (in the routine medium) a medium specially recommended for toxin production by MacFarlane and Knight was tried. The medium consisting of 33% of Bacto-peptone, sulphated horse muscle extract and glucose 0.5 % was prepared and the standard toxigenic strain of Cl. welchii S. R. 12 and strain No. 4 were tested in this medium. It was found that the strains did not yield any toxin. The same medium was tried using different brands of peptone, namely Witte's & Indian peptone but even with these, it was noted that this medium was unsuitable for toxin production. It is likely that the lack of suitable brand of peptone (authors recommend Evan's peptone) might be the cause for its failure.

Since the commencement of the enquiry, material from 22 cases of gas gangrene, became available and it was possible to isolate 14 strains of Cl. welchii either alone or associated with other anaerobes. Out of these, most of the strains of Cl. welchii were tried to study their toxigenic capacity along with the standard strain of Cl. welchii S. R. 12 used as a control. Only one strain of Cl. welchii was found to be toxigenic. The other strains were unable to produce any toxin in the usual medium even when, virulence was increased by repeated animal passage. The control strain and the toxigenic strain of Cl. welchii isolated here, produced toxin lethal to mice on several occasions even though they were kept growing on the laboratory media without passing through animals. Three different media specially recommended for the production of toxin from Cl. welchii have been tried so far to determine the toxigenic capacity of the strains of Cl. welchii.

Nagler's reaction.—The results obtained so far, indicated that the strains of Cl. welchii which failed to yield any toxin in these special media were probably atoxic. The filtrates obtained from these strains were thought to be atoxic by their non-lethal effect on mice when injected intravenously. Nagler has recently described that human serum or egg-yolk saline solution (MacFarlane) brought into contact with a toxin, produce, a turbidy which is a much more sensative index for the α toxin of Cl. welchii type A than its lethal effect on mice. All the strains of Cl. welchii isolated from human cases of gas gangrene are being

tested for this egg-yolk reaction. This study is in progress, and its result will be communicated later on.

IMMUNISATION OF RABBITS.

The work of immunisation of rabbits against toxins of Cl. welchii S.R. 12 and 53, which is undertaken to produce pure antitoxic sera to be used along with experiments in chemotherapy has been continued.

38. Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta.

PROCESSING OF LIQUID SERUM.

The technique employed for the processing of liquid serum remained essentially the same as reported last year, excepting that the " Pilot Seitz Filter " is in use since the 11th February 1943 instead of the 14 cm. seitz filters. This has improved the efficiency of the work very much and it is now possible to filter as much as 80-90 litres in one sitting. The details of the processing of the liquid serum have been communicated in the form of a paper to the Indian Medical Gazette.

From 1st December 1942 to 31st October 1943 ninety-eight batches with a total of 3,552 litres of liquid serum have been processed out of which only one batch of 70 litres was found contaminated and had to be immediately refiltered. The contamination was in all probability due to some faulty pad.

In order to meet the criteria set up by the military for their acceptance of serum the method of taking out sterility test samples was modified as follows :

Three samples are taken out at regular intervals during the filtration. "If the volume of serum filtered is more than 36 litres a sample is taken for every 12 litres or its multiple in such a way that the number of samples never exceeds five. Parallel samples of 5 cc. of serum are run into 50 cc. of glucose broth in transfusion bottles. In case of any discrepancy between the results obtained with the two kinds of samples, those obtained with the former get the preference. If more than 50 % of the former kind of sterility samples, show contamination, and if the bottles of serum are still quite transparent, they are immediately refiltered.

PROCESSING OF DRIED SERUM.

Two drying machines were available : (1) the pilot plant fabricated in the Institute by Dyer, Sankaran and Subramanyan, capable of drying about 4 litres per charge and (2) the Desivac imported from United States capable of drying 25 litres per charge, 14 batches were dried in the first plant and 41 in the second one, during the period under review the total amount of serum dried being 840 litres. Lesser number of batches were dried in the first plant because of the breakdown of the ammonia compressor which has been under repair for 7 months.

Both the above machines were originally meant to dry 250 cc. of serum in special type of bottles, but in order to meet the demands of the military for drying 400 cc. in Bristol type of bottles a bottle of similar height, outside diameter and the inner diameter of the neck, but with straight edge, was designed in co-operation with Messrs. Scientific India Glass Co. The bottle is made of neutral

glass and is capable of standing the wide range of temperature during freezing and drying. Suitable vaccine sleeve type rubber caps are made by Messrs, Bengal Waterproof Co. Even in the above bottle, with 400 cc. the thickness of the shell is more than with 250 cc. hence the drying time is increased from 72 to 94 hours. The moisture content of the dried product is below 1% usually round about 0.5%

The liquid and dried sera produced have been tried in various hospitals and reported satisfactory.

39. Enquiry into the occurrence of ascites in Hyderabad State under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan.

During the period under report 222 cases of ascites with or without oedema and of oedema without ascites were studied. The classification of these under different causes is given in the subjoined tables. The salient points brought out in the study are as follows :—

1. Ascites is seen 2½ times more frequently among males than in females.
2. The cases in which ascites has been provisionally attributed by the writers, to "nutritional" deficiency are very much more frequent among males than females 13.5 : 1
3. The 39 cases of oedema without ascites (17.5 per cent of all the cases of ascites and oedema studied i.e. 222) all occurred among males. If anaemia and beri-beri are also considered nutritional then 20 out of these 39 cases of oedema without ascites are attributable to nutritional deficiency and 18 of these recovered.
4. If all cases of ascites and oedemas are considered together then the cases attributable to nutritional causes (nutritional deficiency, anaemia and beri-beri) number 52 out of 222 i.e. 23.4 per cent the majority of whom are capable of recovery.
5. Nutritional disabilities manifest themselves far more frequently among males than among females. It may be possible that harder labour and exposure in the presence of dietetic insufficiency may play a part in this differential prevalence in the sexes. However, this is not offered as a conclusion.

Amoung the investigations, the following are mentioned :—

1. The rose-bengal test of liver function was done on 20 persons and the results were found to be within normal limits in 3 out of 4 "nutritional" cases but abnormal in all the 12 cases of cirrhosis of the liver. Normal results were also obtained in cases of tuberculosis. This work is being continued.
2. The Takata-Ara test was performed in 112 cases and the results are shown in table V. It shows that in the majority (70%) of cases of ascites attributable to nutritional deficiency, the test is negative, whereas in cirrhosis of the liver the test is positive in more than 80% of the cases. These do not respond well to treatment. *A negative Takata-Ara Reaction thus gives a hope of recovery.*

Classification of the cases of ascites and oedema studied in the Osmania Hospital, during the year 1943, (1st January to the end of November, 1943)

Total number of cases studied			222
Ascites with or without oedema		183	
Males	132		
Females	51		
	183		
Oedema without ascites (all males)		39	
		222	

TABLE I.

Showing the causes of ascites in 183 *cases.*

(*Both sexes*).

Sr. No.	CAUSATION.	No. of Cases.	Percentage of Total (183)	Cured.	Improved.	Discharged otherwise.	Died.	REMARKS.
1. (a)	Cirrhosis of the liver ..	68	37.1	..	5	40	23	
(b)	Syphilitic hepatitis ..	1		1	..	..	..	
2.	Nutritional deficiency ..	27	14.8	15	7	3	2	
3.	Cardiac failure ..	24	13.1	..	13	3	8	
4.	Renal diseases ..	18	9.8	1	3	9	5	
5. Tuberculsis								
(a)	Pulmonary ..	10	9.8	..	3	6	1	
(b)	Abdominal ..	7		..	5	1	1	
(c)	General ..	1		..	..	..	1	
6. Neoplasms								
(a)	Secondaries in the peritoneum	7	7.1	..	..	7	..	
(b)	Stomach ..	1		..	1	..	..	
(c)	Ovarian ..	2		..	..	2	..	
(d)	Rectum ..	2		..	..	1	1	
(e)	Cervix ..	1		..	..	1	..	
7. Other Conditions								
(a)	Anaemia ..	3		..	2	1	..	
(b)	Anaemia and ankylostomiasis	2		..	1	..	1	
(c)	Wet beri-beri ..	2		1	..	..	1	
(d)	Ascariasis ..	2		2	..	..	..	
(e)	Chronic dysentery (amoebic)	2		..	1	..	1	
(f)	Leprosy ..	1		..	..	1	..	
(g)	Acute peritonitis ..	2		..	..	2	..	

TABLE II.

Showing the causes of ascites in male cases—132.

Sr. No.	CAUSATION.	Number of cases.	Percentage of Total 132	Cured.	Improved.	Discharged otherwise.	Died.	REMARKS.
1.	Cirrhosis of the liver ..	51	38.6	..	3	28	20	Diagnosis confirmed on P.M. examination in 6 cases.
2.	Nutritional deficiency ..	25	18.9	13	7	3	2	
3.	Cardiac failure ..	15	11.3	..	7	3	5	
4.	Renal diseases ..	16	12.1	1	3	8	4	
5.	Tuberculosis.							
	(a) Pulmonary ..	8	9	..	3	4	1	
	(b) Abdominal ..	3		..	2	..	1	
	(c) General ..	1		..	..	..	1	
6.	Neoplasm							
	(a) Stomach ..	1	1.5	..	1	..	..	
	(b) Rectum ..	1		..	..	..	1	
7.	Other conditions							
	(a) Anaemia ..	2		..	1	1	..	
	(b) „ Ankyostomiasis ..	2		..	1	..	..	
	(c) Wet beri beri ..	2		1	..	..	1	
	(b) Ascariasis ..	2		2	..	..	..	
	(e) Chronic dysentery (amoebic)	2		..	1	..	1	
	(f) Leprosy ..	1		..	..	..	..	
	Total ..	132						

TABLE III.

Showing the causes of ascites in female cases—51.

Sr. No.	CAUSATION.	Number of cases.	Percentage of Total 51	Cured.	Improved.	Discharged otherwise.	Died.	REMARKS.
1.	(a) Cirrhosis of the liver ..	17	..	..	2	12	3	
	(b) Syphilitic hepatitis ..	1	35.28	1	..	..	..	
2.	Nutritional deficiency ..	2	3.9	2	..	..	..	
3.	Cardiac failure ..	9	17.6	..	6	..	3	
4.	Renal diseases ..	2	3.9	..	..	1	1	
5.	Tuberculosis							
	(a) Pulmonary ..	2	11.9	..	..	2	..	
	(b) Abdominal ..	4		..	3	1	..	
6.	Neoplasm							
	(a) Secondaries in the peritoneum	7		..	..	7	..	
	(b) Ovarian tumor (malignant)	2	21.56	..	..	..	2	
	(c) Rectum ..	1		..	..	..	1	
	(d) Cervix ..	1		..	..	..	1	
7.	Other Conditions							
	(a) Anaemia ..	1		..	1	..	..	
	(b) Acute peritonitis ..	2		..	..	2	..	
	Total ..	51						

TABLE IV

Showing the causes of oedema without ascites in 39 cases (All males)

Sr. No.	CAUSATION.	Number of Cases.	Percentage of Total 39.	Cured.	Improved.	Discharged otherwise.	Died.	REMARKS.
1.	Nutritional deficiency ..	12	51.2	7	3	1	1	
2.	Anaemia ..	7		..	7	..	..	
3.	Beri-beri ..	1		1	..	..	..	
4.	Cardiac failure ..	5	12.8	..	3	..	2	
5.	Renal diseases ..	3	7.6	..	2	..	1	
6.	Pulmonary tuberculosis ..	7	17.9		..	7	..	
7.	Amoebic dysentery ..	2	5.1	2	..	..	..	
8.	Chronic dysentery ..	2	5.1	..	..	2	..	
	Total ..	39						

TABLE V

Results of Takata-Ara reaction in cases of ascites and oedema.
Tests performed=122.

Sr. No.	DISEASE.	Total Cases.	Strong positive.	Positive.	Doubtful.	Negative.	REMARKS.
1.	(a) Cirrhosis of the liver .. (Ascitic fluid)	56	9	37	3	7	Positive in 82.1% of cases.
	(b) Syphilitic hepatitis ..	1	..	..	1	..	
	(c) Neoplasm liver ..	1	..	1	..	..	
2.	Ascites due to malnutrition.						
	(a) Tests performed with serum	7	..	..	1	6	Negative in 70% of cases.
	(b) Ascitic fluid ..	3	..	1	1	1	
3.	Oedema due to malnutrition ..	2	..	..	..	2	
4.	Renal diseases						
	(a) Ascitic fluid ..	6	1	2	1	2	
	(b) Oedema „ ..	2	..	1	..	1	
	(c) Serum ..	3	..	1	1	1	
5.	Abdominal tuberculosis (ascitic fluid) ..	6	..	2	1	3	
6.	Pulmonary tuberculosis						
	(a) Ascitic fluid ..	4	1	2	..	1	
	(b) Serum ..	1	..	..	..	1	
7.	Cardiac insufficiency ..	2	..	2	..	..	
8.	Other conditions.						
	(a) Suppurative peritonitis ..	2	1	1	..	..	
	(b) Neoplasm abdomin ..	1	..	..	1	..	
	(c) Ovarion cystic fluid ..	2	..	..	..	2	
	(d) Amoebic dysentery & ascites	1	..	1	..	..	
	(e) Pleural fluid ..	1	..	..	..	1	
	(f) Hydrocele fluid ..	8	..	..	..	8	
9.	Normal sera ..	3	..	..	..	3	

40. Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

Effect of snake venom on the oxidation of succinic acid in tissue cells.

The biological significance of various organic acids has long been discussed and it has been found that out of a large number of substances investigated, only a few such as pyruvic, lactic and succinic acids increase the oxygen-uptake by tissue cells. The above acids have, therefore, been considered to play a prominent part in the normal metabolism of animals. It has already been reported that snake venoms, specially that of cobra, strongly inhibits the oxidation of lactic acid (as sodium lactate) in the brain cells of pigeons.

In continuation of this work, the effect of cobra venom and its constituents on the oxidation of succinic acid in the brain cells of pigeons has been investigated. The oxygen-uptake of the minced pigeon brain suspended in Ringer-Locke solution with or without the addition of sodium succinate has been estimated in a Warburg's manometer at 37°C. It has been observed that the average oxygen-uptake by brain cells in Ringer-Locke solution alone is 910 mm.^{3} per gm. per hour, while in presence of 0.6% and 0.3% succinate the oxygen-uptake is 1971 mm.^{3} and 1610 mm.^{3} per gm. per hour respectively. The addition of 0.5 mg., 1.0 mg. and 2.0 mg. of cobra venom to the Ringer-Locke-succinate solution reduces the oxygen-uptake by the brain cells to 632 mm.^{3}, 560 mm.^{3} and 510 mm.^{3} per gm. per hour respectively. It is thus evident that cobra venom inhibits the oxidation of succinate to a considerable extent. The effect of some of the constituents of cobra venom like the haemolysin, the choline-esterase and a neurotoxin fraction on this oxidation process has also been studied. They are found not to exert any appreciable inhibting action. It is thus evident that the inhibitor of oxidation of succinate, is not identical with any of the active principles mentioned above.

Effect of snake venom on glycolysis in carcinoma cells produced artificially in rats.

Several batches of rats each consisting of 15 to 20, were injected regularly with carcinogenic hydrocarbons such as methylcholanthrene ; only 12 rats developed tumour after about 3 to 4 months. Using very thin slices (*0.2 mm.*) of these tumour tissues, the conversion of glucose to lactic acid in an atmosphere of nitrogen has been investigated with cobra venom and some of the constituents isolated from it. It has been found that although crude cobra venom markedly suppresses the conversion of glucose to lactic acid, the neurotoxin, the haemolysin and the choline esterase have very little effect on this process of glycolysis. It thus becomes evident that the inhibitor of glycolysis contained in cobra venom is different from the constituents we have, so far, isolated. Attempts were, therefore, made to separate the inhibitor and study its properties. With this object in view, dilute solution of cobra venom has been fractionally precipitated with different salts. It has been found that the precipitate obtained at 50% concentration of $MgSO_4$, $2H_2O$ contains about 95% of this inhibitor. When this substance is dissolved in water and the solution saturated with sodium chloride a precipitate containing about 90% of the inhibitor and about 12% of the neurotoxin is obtained.

ACTION OF COBRA VENOM ON THE SYNTHESIS OF ACETYLCHOLINE IN RAT'S BRAIN.

It is well-known that acetylcholine is synthesised in the brain cells of animals. Quastel and co-workers have shown that this substance exists partly in a combined form and partly in the free state in the brain; the proportion of the combined form being much greater than that of free acetylcholine. We have studied the effect of snake on the synthesis of acetylcholine in the brain cells of rats suspended in glucose-Ringer-Locke solution in an atmosphere of oxygen. It has been found that on an average a total amount of 9.6 of acetylcholine per gm. of rat's brain is synthesised in two hours at 37°C. ; but on the addition of 0.2 mg. of crude cobra venom, the amount of total acetylcholine synthesised is only about 4.0. The inhibition is therefore quite marked. The amount of free acetylcholine on the other hand, is found to increase appreciably on the addition of cobra venom. Evidently this occurs at the expense of the combined acetylcholine since the total acetylcholine (consisting of the combined and the free acetylcholine) is synthesised to a much smaller extent under the latter conditions. The liberation of free acetylcholine from its combined form by the action of cobra venom indicates that the venom contains some enzyme which can effect this splitting. It has also been found that this inhibitor is not identical with any of the fractions such as neurotoxin, haemolysin choline-esterase etc., which have so far been isolated by us from cobra venom.

41. Enquiry into the isolation of atropa from dhatura under Dr. J.N. Ray at the Delhi University Laboratories, Delhi.

The work was undertaken to find out some species of dhatura which may be utilised for commercial preparations of atropa alkaloids. Dhatura is a small shrub and it grows over all parts of India ; but as different species contain different alkaloids of the solanaceous group, and moreover as the alkaloidal content varies considerably with the nature of the soil. Dhatura plants were collected from different provinces of India and subjected to careful examination. Both the black and the white varieties of D. fastuosa, and D. alba as grown in Bengal, U. P., Hardwar, the Punjab and Bombay were examined. Though the percentage of total alkaloid was nearly 0.2 in almost all the varieties, yet the isolation in pure state of either hyoscyamine or hyoscine offered difficulties obviously on account of the presence of some minor alkaloids. Stramonium leaves obtained through the courtesy of the forest officer, the Nilgiris, were also investigated and found to contain 0.3% of total alkaloid, calculated as hyoscyamine. The crude alkaloidal mixture could be fractionated into hyoscyamine and hyoscine but the yields dwindled to 0.05 and 0.01% respectively. Dhatura metal obtained from N.W.F.P. with an alkaloidal content of 0.2% was ultimately found to be the best source of hyoscine and the hydrobromide obtained in 0.05% yield was found to possess all the properties of hyoscine hydrobromide as recorded in literature (M.p. 193-194 (L) D - 24.11).

INTRODUCTION.

Isolation of the solanaceous alkaloids, atropine, hyoscyamine and hyoscine was effected amongst others by Dunstan and Brown in 1899. They have prepared the alkaloids by exhaustive percolation with cold alcohol and subsequent concentration of the percolate under reduced pressure. The alkaloids were then extracted with 0.5% aqueous hydrochloric acid, basified with dilute ammonia and extracted with chloroform. By distilling off the solvent, they obtained

the pure alkaloids and in case of hyoscyamine, they crystallised the alkaloid from chloroform— petroleum ether for final purification. By following this simple procedure of Dunstan and Brown we could not isolate the amm. alkaloids in pure condition from Indian dhatura. Further details of extraction and purification were not available in the literature. By several trial experiments it was found that the temperature of the bath on which the percolate is concentrated should be kept below 45°C in order to prevent the oxidation and resinification of the bases to any appreciable extent. Last traces of alcohol should be removed in order to facilitate the separation of nonalkaloidal resins in the presence of small amounts of acidulated water. The quantity of aqueous hydrochloric acid (0.5%) which is used for dissolving out the alkaloids from the concentrate should be such that an almost complete removal of the alkaloids may be accomplished. If the aqueous acid extract is very dilute or voluminous chloroform will fail to extract out the alkaloid, hyoscine, from the solution after basification with ammonia. In order to ascertain the amount of alkaloid thus left in the mother liquor, the solution was acidified and the whole of the alkaloid was thrown as periodide with Wagner's reagent and recovered by the usual procedure. After several comparative experiments we were successful in finding out the quantity of aqueous hydrochloric acid, required for a definite quantity of dhatura leaves which will minimise the amount of unextractable alkaloid. The aqueous acid extract was then repeatedly agitated with chloroform and ether in order to remove chlorophyll and non-alkaloidal impurities. The solution was then basified and extracted with chloroform. The chloroform extract was slightly coloured and an oil was obtained on the removal of the solvent under reduced pressure. The oil did not even produce the usual red colour with phenolphthalin–a reaction which is characteristic of hyoscine and hyoscyamine. As this loss of sensitivity may very well be due to a decrease in the PH value of the solution resulting from unexpected hydrolysis during removal of chloroform, cold dry ether was added to the oil and at once some solid was found to separate out. This was filtered. The solid was found to be insoluble in dilute acid and was rejected. The ethereal solution on evaporation furnished an oil which also precipitated some non-alkaloidal solid on further dilution with dry ether. By repeating this process of purification thrice, the alkaloid was obtained in pure condition and it responded to the usual colour reactions for atropa alkaloids. Later on this lengthy and tedious process of purification was replaced with advantage by the following procedure. The chloroform extract of the alkaloid was agitated twice with a few c.c. of aqueous hydrochloric acid (1%). This removed the whole of the alkaloid from chloroform layer. The aqueous acid extract was then vigorously shaken at the ordinary temperature with animal charcoal for half an hour. It was filtered and hyoscine was obtained by the usual procedure from this completely colourless solution. Hyoscine was obtained from dhatura metal as a colourless syrup and was converted into the hydrobromide by means of dry hydrobromic acid. This was crystallised from acetone and obtained as a colourless powder (mp. 193-194°(L) D–24.11 literature records mp. 193°(L) D–24° to 26°).

Hyoscine picrate was also prepared, it melted at 187°–188°C. (literature records 187°C) and was also converted into the hydrobromide (mp. 193-194°C) via the free base.

Experimental.

Extractions of the alkaloid.—The solanaceous alkaloids may be extracted by either of the two methods described below. As these alkaloids are very susceptible to heat which either hydrolyses or recemises or resinifies them, the cold extraction method was first pursued but later on it was replaced by the second method with advantage.

The first method.—Finely powedered leaves (500G) were taken in a litre flask and 2000 c.c. of a mixture of 4 volumes of ether and 1 volume of alcohol was added. It was shaken well for 4 (four) hours and set aside for 1/2 hour and 60 c.c. of dilute ammonia solution added and shaken frequently during an hour. The mixture was then transferred to a separating funnel plugged with cotton and when the liquid ceased to flow, it was packed firmly and percolation continued first with 250 c.c. of a similar mixture of alcohol and ether and then with ether for about two hours. To the percolate was added 800 c.c. N/2 HCL shaken well and allowed to settle. This aqueous acid extract was neutralised with dilute ammonia using litmus as indicator. The solution was then evaporated in vacuo to 400 c.c This was then basified with ammonia and extracted 12 times with chloroform. On distilling off the solvent the crude alkaloid was obtained.

The second method.—In this experiment we describe the isolation of hyoscine from the dried leaves of D. Metal obtained from N.-W.F.P.

Powdered leaves (8 lbs.) were exhausted by percolation with cold rectified spirit and the percolate was concentrated under reduced pressure from a water bath (temperature 45°C). When the concentrate was freed from the last trace of alcohol, it was agitated with warm (50°C) water (200 c.c.) and finally with 0.5% aqueous hydrochloric acid (200 c.c.). The extract, thus obtained, was shaken up with ether and chloroform to remove chlorophyll and non-alkaloidal impurities. The solution was deep red in colour, was cooled in ice and basified with a saturated solution of sodium bicarbonate and extracted six times with chloroform. The combined chloroform extract was once washed with water and then extracted with a few c.c. of 1% hydrochloric acid. This extract was slightly yellow in colour and it was completely decolourised with charcoal. This was filtered from charcoal basified with sodium bicarbonate solution and extracted again with chloroform. The chloroform extract was completely colourless, it was well washed and kept overnight over anhydrous sodium sulphate. On removing the solvent, hyoscine was obtained as a colourless mobile liquid which was identified by the following reactions.

The following experiments were conducted for ascertaining the nature of the alkaloid.

(a) The alkaloid was found to colour a phenolphthalein paper red when tested in the following way.

A minute quantity of the syrup was placed on a piece of phenolphthalein paper and then wetted with strong alcohol, no colour appeared. The alcohol was next allowed to evaporate and on touching the alkaloid with a drop of water, a red colour appeared. This reaction is common to hyoscyamine and hyoscine but is not given by any other alkaloid commonly used.

(b) Gerrard's test.

0.1 G of the base was placed on a watch glass and few c.c. of a 2% solution of mercuric chloride in alcohol was added. Neither any colouration nor any precipitate was produced. So the base must be hyoscine as with atropine and hyoscyamine a red colouration is obtained.

(c) Vitali's reaction.

0.1 G of the base was taken in a basin and treated with a drop of fuming nitric acid and evaporated at 100° C. The residue, when cool, was touched with a freshly prepared solution of potassium hydromide in absolute alcohol. A violet colouration was produced, which slowly changed to dark red and ultimately

disappeared but it could be reproduced by adding more of alcoholic caustic potash. This colouration is peculiar to the atropa alka.oids only.

PREPARATION OF THE SALTS.

Hyoscine hydrobromide.—The usual method of neutralisation of the base with dilute hydrobromic acid failed owing to the solubility of the salt in water. But when an ethereal solution of the base was cooled in ice and a cold ethereal solution of anhydrous hydrobromic acid was added dropwise to it, the hydrobromide was obtained as a white precipitate. This was filtered and finally crystallised from acetons. The hydrobromide was thus obtained as a white powder (mp. 193-194°C (L) D- 24.11).

The picrate was prepared by adding an ethereal solution of picric acid to an ethereal solution of the base. This when twice crystallised from absolute ethylalcohol melts at 187-188°C. This, when depicrated with ammonia in ether, furnished hyoscine from which the hydrobromide (mp. 193-194°C (L) D-24.11) was obtained.

The results of the investigation on the different species of dhatura collected from different localities are shown in the table below :—

Name of the species.	Localities collected from.	Result of investigation.
1. Dhatura Fastuosa v. niger	(i) Bengal. (ii) Delhi. (iii) Hardwar. (iv) Punjab (v) Bombay.	0.2 to 0.3% total alkaloids. Both hyoscyamine were found to be present along with a considerable amount of minor alkaloids.
2. Dhatura alba	(i) Bengal. (ii) Delhi. (iii) Hardwar. (iv) Punjab.	0.2 to 0.3% total alkaloids. Both hyoscyamine were found to be present along with a considerable amount of minor alkaloids.
3. Hyoscyamus v. niger.	U.P.	0.02% of the total alkaloid on account of this poor yield was not worked up for extraction of alkaloids in pure state.
4. Dhatura stramonium.	Madras.	0.3% of total alkaloid contains hyoscyamine as well as hyoscine in 0.05 and 0.01% respectively.
5. Dhatura metal	N.W.F.P.	0.2% of total alkaliod yields pure hydrobromide of hyoscine in 0.05% yield. It may be regarded as the best source of hyoscine, so far as Indian raw materials are concerned.

Thus it has been found that dhatura metal may serve as a source of hyoscine and hyoscine hydrobromide for the commercial preparation of the drug.

42. Leptospirosis enquiry under Dr. M.N. Lahiri at the Haffkine Institute, Bombay.

The work carried out may be classified under the following heads :

Human cases of leptospirosis.

During the period under review fifty-six cases of acute jaundice with fever were studied. Besides, a fair number of cases were also referred to the enquiry but these have not been taken into account in view of the fact that the symptoms were of a chronic nature. Out of those fifty-six cases eleven proved to be authentic cases of leptospirosis and in each instance the diagnosis was established by laboratory investigations. Two of these positive cases had a fatal termination. In one, autopsy was performed and no leptospirae could be demonstrated in the liver and kidney sections stained by Levaditi's silver impregnation method. This is in consonance with the findings of other workers. In addition to the above two, there were four more fatal cases in the series and in two of these, however, the symptoms were very suggestive of leptospirosis but the patients died before any material could be obtained for examination. The causal organism was isolated from six of these positive cases and in the remaining the diagnosis was based on agglutination and other serological tests.

For the isolation of the organism the useful method of blood culture could not be employed in any of the cases as either the disease was not suspected or the cases came under observation at a time when the infective period of the blood had passed. For the isolation, therefore, the daily systematic inoculation of urine into guineapig proved to be a very useful procedure although it involved a good deal of labour and the use of a large number of animals. For this purpose, on an average eight samples of urine were used from each case between the tenth and twenty-first day of illness. It was observed that in every case only one sample of urine proved to be infective except one where two samples consecutively produced the infection in animals. This suggests that leptospirae are excreted out intermittently or that the samples containing the organisms may fail to produce infection. Further, certain animals here were found to be refractory to infection. The strains isolated all belonged to the "classical" group. The agglutination reaction proved to be a very useful method. In fact the diagnosis was returned much earlier even in the cases from whom the organisms were isolated. In view of the previous experience in carrying out the tests the strains isolated locally were used. The tests were repeated not only to note the fluctuation of titre but also the end of titre. From each case three samples of sera were examined and the last one usually was collected at the end of the third week after illness.

Cases without icterus.

The first case without jaundice was reported last year. This year the physicians referred six such suspected cases but all proved to be negative by laboratory studies. With a view to shed further light on the incidence of such form of the disease, samples of sera were examined for the presence of agglutinins which were sent to this Institute for routine serological reactions. Only those sera were picked out which gave negative widal reaction against enteric group of organisms and at the same time clot culture also was found to be negative. In all, one hundred and fifty specimens of sera were tested out of which one showed a titre of 1:640. In carrying out the tests three local strains were used. The positive serum was not bile-tinged at all. This indicates that the case must

have been incorrectly diagnosed. It is suggested that cases of acute febrile illness which resembles influenza or enteric fever, the physicians should be alert to confirm or exclude the diagnosis of leptospirosis.

Infective hepatitis.

Towards the later part of the year a number of cases of jaundice were noticed in a locality of this city. The initial symptoms i.e. shivering attacks, headache and body pains in some of them were suggestive of leptospirosis clinically but the infection was ruled out by laboratory studies. The examination of blood smears showed no parasites or any abnormality. Agglutination tests against enteric group of organisms were negative. Vanden Bergh reaction was immediate direct positive. With regard to age incidence persons at all age were effected. There was only one case in each family but in one three brothers suffered from marked jaundice one after another with an interval of about a month. This suggests the infective nature of the disease. Regarding mode of spread particularly in this family, it is not improbable that the infection might have been spread by droplets. It is proposed to carry out a detailed study of such cases.

Medium.—In carrying out agglutination tests it was found advantageous to use as antigen cultures free from agar. For this purpose Vervoot's medium was used but some difficulty was encountered in obtaining good growth in some batches of the medium although prepared in an identical manner and some even would not sustain growth. As individual animals vary considerably in the suitability of their sera for the cultivation of leptospira pooled serum was always used. It was shown by Zimmermann that a loopful of the sterile culture of yeast in a medium considerably improved the growth. Few observations carried out, confirmed this finding. Yeast is the rich source of vitamin B-complex and nicotinic acid is an important member of it. Since nicotinic acid has also played an important role in bacterial metabolism a series of experiments were carried out to demonstrate if it had any enhancing effect on the growth of leptospira. It was observed that concentration at 0.01 per cent gave the best growth and the concentration above this optimum level had an adverse effect.

Riboflavin another member of the vitamin B-complex was also tried but from a few observations no beneficial effect was noticed even at concentration of 0.005%. As the substance is expensive and also difficult to procure experiments had to be discontinued and no definite conclusions could therefore be arrived at. Besides, the following amino acids, glutamic acid, tyrosine and glycine instead of peptone were also incorporated in the medium but none so far had an enhancing effect on growth, on the contrary last one markedly supressed the growth of the organisms. Now a simple media has been evolved as follows : 10% pooled rabbit serum in distilled water PH 7 is heated to 56°C. for half an hour. Nicotinic acid solution of the strength 1% in normal saline is prepared and is sterilised by filtration through L3 candle. This solution once prepared can be stocked in a cool place for some time. The necessary quantity of this solution is finally added to the distilled water serum mixture to obtain the concentration of 0.01%. Preliminary studies have given encouraging results and it is proposed to use this medium on a large scale along with the Vervoot's medium for comparison. Further attempts will also be made to improve the medium.

Transmission of leptospira to mice.

It was reported that the Haffkine Institute inbred young mice were fairly resistant to the strains of leptospira isolated here. From the laboratory experiments of yellow fever virus on mice it is reasonable to assume that different

strains of mice might also vary in their susceptibility in the case of leptospira also. For this reason Javanese strain of mice were used and only mice three week's old were employed. Varying doses of two recently isolated cultures ranging from 0.2 to 0.5 were used. For each dose three mice were taken along with a young guineapig as a control. Out of 30 mice used in these experiments only two showed evidence of infection and the organism could be recovered by culture of the heart's blood. The control guineapig in every instance succumbed to the infection following intraperitoneal inoculation with one c.c. of the test dose of the cultures. The Institute is now breeding Swiss strain of mice and an attempt will be made to ascertain the suitability of this strain for experimental infection. So far these experiments do not lend support to Larson's findings but it is not known if he has been using any particular strain of mice.

Miscellaneous investigations.

Nine samples of convalescent sera from cases and also a specimen of anti-leptospiral rabbit serum were dried from the frozen state by the cryochem method. These dried sera were stocked in the refrigerator. After an interval of two months contents of the ampoule from each specimen of serum were rehydrated and their agglutination titre was determined. For this purpose the same strains of Leptospira were used throughout. It was noticed that even after the lapse of nine months there was no significant drop in their titre. In fact, in all, titre almost approached the original titre. For storing diagnostic serum this method appears to be very useful.

The serum from the personnel of the rat establishment of this Institute were examined for the presence of immune bodies. These persons have been handling rats caught in different parts of the city for a number of years and are exposed in their work to rat excreta. None showed any evidence of the presence of agglutinins. This shows that the factor of chance plays an important role in the transmission of the disease.

REPORTS OF ADVISORY COMMITTEES.

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 10 a.m. on December 13, 1943. It was constituted as follows :—

Brigadier G. Covell (*Chairman*).

Lieut.-Col. M. K. Afridi.

Dr. Anantaswami Rao.

Brevet-Col. Sir Ram Nath Chopra.

Dr. B. M. Das Gupta.

Lieut.-Col. H. W. Mulligan.

Major Jaswant Singh (*Secretary*).

PROCEEDINGS.

The Committee considered the report on malaria enquiries carried out during the current year and the proposals for 1944-45.

EXISTING ENQUIRIES.

1. *Malaria Institute of India* (*Research Section*).

Research activities have again been greatly curtailed owing to abnormal conditions brought about by the war. Five courses in malariology have been held during the year at which 124 officers have received training. The majority of these were medical officers of the Defence Forces, the remainder being Cantonment Antimalaria Officers or Assistants.

An extensive series of experiments has been carried out on insecticides and mosquito repellents. A standard emulsifier locally prepared has proved biologically stable for a period of 9 months, even after dilution with water. The anti-mosquito spray prepared from this, which contains 0.025 per cent pyrethrins, has been used extensively during the past malaria season in Delhi and has proved effective against flies as well as mosquitoes.

The standard type of hand sprayer (MISH) has been modified in certain respects to attain greater simplicity.

Of the larvicides tested, cuprous cyanide proved twice as toxic to anopheline larvae as paris green, applied either as a dust or by dustless method. A mixture of used engine oil 90 parts, kerosene oil 2, diesel oil 5 and Attock antimalarial oil or malariol 3 has been found effective.

Laboratory research has been restricted mainly to the preparation of antisera for determining the source of mosquito blood meals. 5,731 tests have been carried out by this method. A water soluble stain prepared from materials available in the local market has given satisfactory results. Field researches in certain villages near Delhi with special reference to the spray-killing of adult mosquitoes have been continued. The antimalaria operations in Delhi have again been supervised by the staff of the Institute. The study of mosquito bionomics has been continued and the routine identification of mosquitoes and other insects of medical importance has been carried out as in former years.

The research unit operating in North Kanara completed a year's investigation on June 1 and has now been withdrawn.

SOUTHERN INDIA BRANCH.

Mosquito infectivity experiments with *P. knowlesi* and *P. gallinaceum* were continued. No mosquito infections were obtained with *P. knowlesi*. Six species of *Aedes* were infected with *P. gallinaceum*. A study of the eggs of *A. fluviatilis* has shown that the two different types of egg patterns previously observed are not hereditary in character.

A number of studies on the bionomics of *A. fluviatilis* have also been carried out. A laboratory colony of this species has been successfully established. Colonies of *A. stephensi*, *P. gallinaceum*, *Aedes albopictus* and *Armigeres obturbans* have been maintained.

PROPOSALS FOR 1944-45.

(a) *Delhi.*

1. Systematic testing of insecticidal sprays, mosquito repellents and larvicides and spraying apparatus.

2. Testing of the therapeutic effects and toxic action of drugs as evidenced by their effect on animal malaria as occasion demands.

3. Intensive study of bionomics of mosquito colonies maintained in the laboratory.

4. Determination of food preferences of various species of mosquitoes by the precipitin test.

5. Attempts to increase the efficiency of the various antisera employed for precipitin tests.

6. Observations on the bionomics of *A. culicifacies.*

7. Epidemiological studies on malaria conditions in certain villages and coolie camps.

(b) *Coonoor.*

1. Observations on the bionomics of *A. fluviatilis* and other mosquito species of the Nilgiris foothills.

2. Systematic testing of insecticidal sprays, repellents, larvicides and spraying apparatus, both under laboratory and field conditions.

3. Maintenance and study of *P. gallinaceum* and of mosquito colonies.

2. *Pharmacological enquiry at the Haffkine Institute under Dr. B. B. Dikshit.*

(i) *Studies on synthetic antimalarial drugs.* A number of drugs of the sulphonamide group have been tested on monkeys infected with *P. knowlesi*. Three of these were found to have antimalarial properties. 2-N'-sulphanilamido-5-isoprophylthiazole was the most active and was also the least toxic.

(ii) *Studies of immunity in monkey malaria.* Further work was carried out with splenic extracts, which were found to vary very considerably in their effects, some of them having very little action whilst others conferred a certain degree of immunity on normal monkeys.

PROPOSALS FOR 1944-45.

It is proposed to continue the investigation on similar lines.

3. *Filariasis enquiry under Dr. Sundar Rao.*

(i) *Morphology of filariae from different areas in India.* Infections in Damda (Drug District, C.P.) are exclusively *Wuchereria malayi.*

Microfilariae of *W. bancrofti* originating from various localities in Bengal, Orissa and U.P. showed no difference in morphology or measurements.

Filarial infections in dogs and horses have been studied.

(ii) *Studies on the periodicity of microfilariae.* Hydrocele fluid containing microfilariae was inoculated into the hydroceles of 4 filariae free subjects. In two of these cases microfilariae were demonstrated for varying periods.

(iii) *Studies on filarial allergen.* The globulin fraction has been isolated. Further work is in progress to determine its value in treatment.

(iv) *Treatment of filarial infection.* None of the numerous drugs administered to patients suffering from filariasis proved of any value.

(v) *Other observations.* In a limited number of experiments, mixed filarial vaccine appeared to enhance the bacteriolytic power of the sera of filarial patients. The pH of 25 clear hydrocele fluids was found to range between 7.4 and 7.8. The pH of the sweat of 10 filarial patients ranged from 4.5. to 6.0. The Wasserman reaction of hydrocele fluid has been found to give a positive reaction in cases where the blood was also positive.

PROPOSALS FOR 1944-45.

It is proposed to continue the investigation on similar lines.

PROPOSALS FOR NEW ENQUIRIES.

1. *Statistical enquiry into the method of forecatsing epidemic malaria under Dr. M. Yacob.*

It is proposed to study the basic data from which forecasts have been prepared of previous malaria epidemics in the Punjab during the past 50 years and by calculating regression and trends to develop the method further so as to place it on a sounder basis and eliminate the personal factor.

2. *Enquiry on substitutes for quinine and emetine under Dr. B. Mukerji.*

It is proposed to analyse a number of indigenous drugs and to try their effect on the malaria of monkeys and birds and subsequently on cases of human malaria.

Investigation of certain indigenous drugs reputed to cure dysentery will also be carried out on similar lines.

The Committee made the following recommendations :—

EXISTING ENQUIRIES.

1. *Malaria Institute of India (Research Section) and Southern India Branch.* The Committee approved the proposals put forward but expressed the opinion that the existing staff is insufficient to cope with the volume of work which it is now called upon to perform. They recommended that the two posts of Malaria Research Officers at present held in abeyance should be filled as soon as suitable candidates become available.

2. *Pharmacological enquiry at the Haffkine Institute under Dr. B. B. Dikshit.* The Committee recommended the continuance of this enquiry.

3. *Filariasis enquiry under Dr. Sundar Rao.* The Committee recommended the continuance of this enquiry.

PROPOSALS FOR NEW ENQUIRIES.

1. *Statistical enquiry into the method of forecasting epidemic malaria under Dr. M. Yacob.* The Committee supported the proposals put forward.

2. *Enquiry on substitutes for quinine and emetine under Dr. B. Mukerji.* The Committee did not support this enquiry.

REPORT OF THE NINTH MEETING OF THE NUTRITION ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 1st and 2nd OCTOBER, 1943.

COMPOSITION OF THE COMMITTEE.

Lt.-Col. E. Cotter, I.M.S., Public Health Commissioner with the Government of India. (*Chairman*).

Professor S. P. Agharkar, Professor of Botany and Head of the Botany Department, University College of Science, Calcutta.

Dr. K. P. Basu, Lecturer in Chemistry and Incharge of the Biochemical Laboratory, University of Dacca, Dacca.

Khan Bahadur Dr. A. H. Butt, Director of Public Health, Punjab, Lahore.

Dr. B. C. Guha, Ghosh Professor of Applied Chemistry, University College of Science and Technology, Calcutta.

Dr. V. N. Patwardhan, Asst. Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Dr. G. Sankaran, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Mr. J. Sargent, Educational Adviser to the Government of India.

Mr. H. R. Stewart, Agricultural Commissioner with the Government of India.

Dr. W. R. Aykroyd, Director, Nutrition Research Laboratories, I. R. F. A., Coonoor. (*Secretary*).

The following were present by invitation :—

Major-General Sir John Taylor, I.M.S., Director, Central Research Institute, Kasauli.

Major A. M. Thomson, D.A.D.M.S. (Nutrition) Medical Directorate, New Delhi.

Dr. M. B. Daver, Nutrition Officer, Medical & Public Health Department, Hyderabad-Deccan.

Dr. Mohd. Musa Khan, Nutrition Officer, Public Health Department, Punjab, Lahore.

Dr. K. Mitra, Officer-in-Charge, Nutrition Scheme, Public Health Laboratories, Bankipore, Patna.

Dr. K. C. Sen, Officer-in-Charge, Animal Nutrition Section, Imperial Veterinary Research Institute, Izatnagar.

The Committee met on the 1st and 2nd October, 1943 and considered the following :—

1. THE ORIENTATION OF NUTRITION RESEARCH.

At the previous meeting of the Committee in December, 1942, Dr. V. N. Patwardhan suggested that the Committee should take stock of the present position in nutrition research in India and formulate a policy with regard to the kind of nutrition research that should be recommended to the Association for future financial support. In October 1943 the Committee surveyed the existing position of research in various fields and indicated the gaps in existing knowledge which require to be filled. It also suggested various relatively

undeveloped or new lines of research to which, in its opinion, special attention should be given. Its conclusions, given in the sections which follow, will serve the double purpose of guiding the Committee itself in its future deliberations and drawing the attention of university authorities, research workers, and others in India to the fields of nutrition research in which at the present time development is desirable and likely to prove most fruitful.

Basic research.—The Committee recognised that no clear-cut distinction could be drawn between " basic " and *ad hoc* or applied research with an immediate, obvious and direct bearing on problems of human nutrition in India. It did not feel that it would be useful or desirable to make a rigid apportionment of funds to research of any particular type. Basic research must, however, be supported in order to provide the tools for applied and *ad hoc* research and to help in maintaining high scientific standards in all fields of research. As far as possible, however, basic research should be related to practical problems. It should be entrusted to experienced workers with adequate facilities and young workers entering the field should be associated with laboratories where skilled and experienced direction is available. Certain subjects on which, in the Committee's opinion, basic research is particularly needed are indicated in various subsequent sections.

2. The analysis of foods.

A considerable amount of work on this field has already been carried out, the results of which will be found in Health Bulletin No. 23 " The nutritive value of Indian foods and the planning of satisfactory diets " and other publications. The Committee expressed the opinion that further work should include the following :—

(a) The more complete analysis of important and commonly consumed foods, with special reference to the vitamin B complex and other vitamins, to fill in gaps in existing knowledge.

(b) The complete analysis of foods less widely and abundantly consumed, available in different provinces and of local importance, which have not yet been studied.

(c) Further analysis of important *varieties* of cereals which are commonly consumed, as well as new strains which agricultural departments have evolved and propose to distribute (this might include the study of new varieties of wheat tested for milling and baking qualities in the Government Agriculture College, Lyallpur).

(d) Study of the effect of different methods of cooking in order to discover the nutritive value of foods as actually consumed.

(e) The investigation of " trace elements " in foods.

3. The absorption and utilisation of proteins, fats, calium, iron and other food factors by human beings.

The chemical analysis of foods and diets may not provide fully satisfactory knowledge about their nutritional value for human beings. There is an interaction between various food factors and the absorption and retention of any particular constituent may be influenced by the nature of the diet and its composition as regards other constituents. Progress has already been made in the study of problems in this field by investigations on human beings and the Committee recommended that such work should be continued and developed. As far as

possible it should be done on human subjects, but there is still room for the employment of rats and other experimental animals in approaching various problems. The Committee asked one of its members to prepare a review on the subject which should summarise results already obtained for the guidance and information of research workers proposing to undertake work in this field and Dr. K. P. Basu kindly agreed to undertake this task.

4. Basal metabolism.

A considerable number of investigations on this subject have been carried out in India. These have in general shown that the basal metabolic rate of healthy Indian subjects is on the average below that of European or American subjects. No satisfactory explanation of this difference has hitherto been forthcoming. The Committee decided that research which consisted of the mere uncritical collection of further basal metabolic readings could not justifiably be supported. On the other hand research into the influence of racial, dietetic and climatic factors on basal metabolism would be of value, and should be supported. The Committee asked one of its members to prepare a critical review of the present state of knowledge of the subject for the benefit of research workers interested in this line of research. Dr. V. N. Patwardhan kindly agreed to prepare this review.

5. Diet surveys.

About 100 diet surveys have been carried out in various parts of India ; these have provided a reasonably adequate picture of dietary habits in many parts of the country. Large areas, however, remain in which no surveys have been made. These include Bombay (apart from Bombay City) Sind, U. P., and N.-W.F.P.

The Committee expressed the view that there is need for further diet surveys, e.g. in areas not hitherto surveyed. Re-surveys of areas should be carried out after an interval in order to discover whether any changes in diet have taken place. The Committee felt that the undertaking of dietary surveys should be largely the responsibility of provincial nutrition officers, to whom reference will be made in a later section.

In most surveys intake of food has been assessed by weighing uncooked foods. The Committee recommended that attention should be given to enquiries in which the nutritive value of foods as actually consumed after cooking is determined. These would indicate with a greater degree of accuracy the actual intake of the various essential food-factors.

Diet surveys show what foods are consumed by a particular sample of the population. They do not necessarily elucidate the underlying reasons which determine what foods are consumed. Some valuable work had been done on the economic aspects of the problem both by nutrition workers and workers engaged in economic enquiries. While the amount of money available for expenditure on food is normally the most important factor in determining the nature of the diet, there are others which cannot be disregarded. The Committee was of the opinion that the socio-economic and psychological background of dietary habits and prejudices requires investigation. For example, it is sometimes found in diet surveys that groups do not make use of some easily available, cheap and nutritious food. This may be due to ignorance of how to prepare and cook such food in palatable form or to other less immediately obvious reasons. The Committee realised that the methods to be followed in

studying such questions differ from the familiar methods of nutrition research and that investigators with unusual qualities will be needed. It nevertheless emphasised the importance of the subject as an aspect of the problem of nutrition in India and suggested that ways and means of studying it should receive careful future consideration.

6. State of nutrition and malnutrition.

The related problems of devising suitable methods of assessing state of nutrition and detecting malnutrition have been studied with recent years by numerous workers in many countries including India. Numerous lines of approach, clinical, anthropometric, physiological and biochemical have been followed but there is no general agreement as to the most reliable method or methods and no single method has received general approval. The subject is one of great importance in the practical approach to nutrition problems.

The committee, while fully realising the difficulties, recommended the continuance of work in this field. Investigators should bear in mind the objective of devising methods which would be generally applicable in practice. Basic research on specific deficiency states should be prosecuted. It expressed the view that no application for work in this field should be considered unless it is clear that the applicant has studied and understands the vast literature of the subject and realises its difficulties.

Owing to racial and other differences it is impossible to establish general height and weight standards which could be used throughout the country in assessing physical development or state of nutrition. It would be of value to collect height, weight and other anthropometric data in relation to age, sex and race based on the examination of selected groups of children in a good state of nutrition *e.g.* children in well-run boarding schools or schools attended by children of the well-to-do classes. The state of health in such groups should also be assessed clinically. Comparisons can then be drawn between such children and other children (*e.g.* poor children) in the same area suspected of being in a state of malnutrition. The data obtained by the examination of well-to-do healthy children provide a local standard or yardstick by which the degree of malnutrition in other children in the same part of the country can be assessed.

7. Clinical research.

The Committee recognised the great need for clinical research in the field of nutrition in India and considered ways and means of developing it. Because of the great mass of clinical material available workers in India have the opportunity of leading the world in this line of research. The special importance of research into children's and ophthalmic diseases from the nutritional standpoint was recognised. The Committee was in agreement that the establishment of clinical research units would further the satisfactory development of clinical nutrition research.

It was decided that a section from a report by Dr. W. R. Aykroyd, showing the many clinical problems which require study and the extent of the field to be covered, should be circulated to Deans of Medical Faculties, Principals of Medical Colleges and Directors of Laboratories with the suggestion that applicants for Medical Research Fellowships under the I. R. F. A. should consider the possibilities of taking up work on some of the subjects listed. The response would guide the Committee in considering plans for the future development of clinical nutrition research. The passage in question is as follows :

Research on disease: There are numerous diseases in India which are directly or indirectly associated with diet. Some of these may be roughly classified as follows :

(1) Diseases directly due to lack of vitamins or other food factors :
   (a) Vitamin A deficiency—keratomalacia, xerophthalmia and night-blindness.
   (b) Vitamin B deficiency—beri-beri and infantile beri-beri.
   (c) Vitamin B_2 deficiency—pellagra and other conditions associated with nicotinic acid deficiency, angular stomatitis oro-genital syndrome, superficial keratitis and other eye conditions.
   (d) Vitamin C deficiency—scurvy.
   (e) Vitamin D deficiency and calcium deficiency—rickets and osteomalacia.

Goitre and nutritional oedema can probably be included in this group.

(2) Diseases associated with malnutrition.
   The anaemias and especially the anaemias of pregnancy.

(3) Diseases which are associated with some toxic factor in the diet :
   (a) Lathyrism.
   (b) Epidemic dropsy.
   (c) Fluorosis.

(4) Various diseases in the causation of which unsatisfactory diet is probably concerned.
   (a) Peptic ulcer.
   (b) Dental caries.
   (c) Infantile biliary cirrhosis and perhaps certain forms of chronic cirrhosis in adults.
   (d) Urinary lithiasis.
   (e) Diabetes.
   (f) Tropical ulcer.
   (g) Sprue and certain forms of chronic diarrhoea associated with malnutrition.
   (h) Eclampsia.

(5) Diseases of infective origin, the causation and epidemiology of which may be influenced by the nutritional factor.
   (a) Respiratory diseases (particularly in infancy and childhood)—Tuberculosis.
   (b) Leprosy.
   (c) Dysentery and other intestinal diseases.

This list is far from exhaustive. In any Indian hospital one can observe numerous obscure conditions of quite unknown etiology which may be associated with defective diet.

A good deal of research has been carried out on most of the vitamin deficency diseases included in group 1, but much remains to be done. Goitre has

received little attention since the time of Sir Robert McCarrison, though it is a most serious public health problem in large areas of Northern India. (Recently a preventive experiment on goitre in Kangra has been undertaken by the Nutrition Officer in the Punjab). The anaemias (2) have been extensively studied by Napier and his co-workers, but knowledge of their etiology, etc. is still far from complete. Among the diseases in group (3), epidemic dropsy has received considerable attention, and a beginning has been made with the study of fluorosis of the teeth and bones. Lathyrism is a famine disease and of rare occurrence during recent years.

Group (4) : Some investigations on peptic ulcer have been made by surgeons directly concerned with the disease. A preliminary survey of incidence has been carried out by Dogra. There have been a few surveys of the incidence of dental caries in various parts of India, which have shown that Indian children suffer much less from caries than European and American children. If the reason for this difference could be discovered we should be a long way nearer the elucidation of the cause of what has been described as the commonest of all diseases. The etiology of various forms of hepatic cirrhosis in children and adults is quite obscure ; various theories that they may be nutritional in origin have been put forward. Stone in the bladder has been studied by McCarrison, but by no one else. The cause of diabetes—the scourge of the middle classes—is not fully understood and little work has been done on the subject in India. The relation between tropical ulcer—a very common condition among plantation labourers—and diet deficiency requires further study. Sprue and a variety of sprue-like diseases, unquestionably associated with the dietary factor, require further investigation. Nothing definite is known about the cause of eclampsia and whether it can be prevented by suitable regulation of the diet.

As regards (5) (a) attention may be given to the conclusion of de Haas, working in Java, that " the frequency and fatality rate of broncho-pneumonia and the general state of nutrition of children are in closer interrelation than is the case in the majority of other infectious diseases." That there is a close association between malnutrition and tuberculosis is generally accepted, but the formidable tuberculosis problem in India has not yet been studied from this angle. Some preliminary work has been done on the relation between leprosy and nutrition, but so far no definite conclusions have been reached. As regards (5) (c), we have the fact that in times of food shortage and famine there is invariably a striking increase in the incidence of, and mortality from, intestinal diseases described as " dysentery and diarrhoea ".

Apart from specific diseases, there are certain branches of clinical medicine in which research to determine the importance of the nutrition factor is particularly required. These include :

(a) Diseases of infants. Causes of infantile mortality.

(b) Diseases of children aged 1-10. Some 50 per cent of all deaths in any given year in India occur in children below the age of 10 years and it is clear that malnutrition plays an important part in producing this high mortality.

(c) Diseases of pregnancy and lactation. Causes of maternal mortality.

(d) Ophthalmic diseases. Evidence is accumulating that the nutritional factor is of great importance in this branch of medicine.

It is clear that there is a great need for research on disease in India from the nutritional angle. In the case of a number of diseases mentioned above, little accurate knowedge exists about geographical incidence, age and sex incidence, social incidence, etc.

The collection of such data by a preliminary survey is of genuine value. It is to be observed that for this purpose the mere inspection of hospital records or correspondence with medical officers in charge of hospitals is unlikely to provide accurate information. The investigator must adopt a more critical approach and make personal visits and enquiries. As an example of a useful preliminary epidemiological enquiry, the investigations on peptic ulcer recently carried out by Dogra may be cited.

In my opinion the Committee should encourage preliminary survey work of this kind on diseases which are suspected to be, directly or indirectly, nutritional in origin. Such investigations must, however,.be in the hands of individuals who are both experienced in clinical medicine and have at the same time some knowledge of scientific and statistical methods.

It has been remarked that the diseases of infancy and childhood, the diseases of pregnancy and lactation and ophthalmic diseases should be the object of research from the nutritional angle. A busy clinician engaged in private practice can rarely undertake detailed research, while a young doctor who is eager to make original contributions to medicine has often insufficient understanding of scientific method. If progress is to be made in elucidating the relation between the diseases of infancy and childhood, etc., and the nutritional factor some other method must be found besides the giving of occasional grants to workers who wish to study some limited aspect of these various subjects. It is suggested that the creation of *clinical research units*, attached to a few of the larger hospitals in India, and in particular hospitals for children and ophthalmic diseases, would be a step in the right direction. It would also be in conformity with the practice in other countries. A discussion of the personnel of such units, and their relation to hospital staffs and research institutes would be beyond the scope of this note. It is to be observed that non-medical workers, e.g. biochemists, could play a valuable part in the work of such units.

With regard to clinical research in general, the following important point should be noted. In undertaking laboratory research, the research worker in India competes on equal terms with his colleagues in England and U.S.A. He has the same opportunity for making original contributions to science, if he is working in a well equipped laboratory. But when he undertakes research on nutritional disease in India then his opportunities for making original observations of striking interest and value are far greater than those he would find in England or U.S.A., for the simple reason that there is more disease, and a greater variety of disease, associated with faulty diet in India. There is a remarkable abundance of " clinical material " awaiting investigation. Virgin soil is more fruitful than soil which had been intensively cultivated."

8. Conjoint research.

The practice of carrying out research on special problems by means of investigations in which several laboratories are associated is one which should be followed. This would be specially valuable in the case of controversial subjects and subjects of All-India importance. Collaboration with agricultural and animal nutrition laboratories will be desirable in many instances. Subjects which might be studied in collaboration with the latter include :

(a) The vitamin content of milk as influenced by season, fodder, etc.

(b) The availability of calcium in leafy vegetables.

(c) Fluorosis in man and domestic animals.

(d) Rich sources of pro-vitamin A (carotene).

9. THE ORGANISATION OF NUTRITION RESEARCH.

The Committee considered that the present system of supporting nutrition research by temporary year to year grants to individual workers has various disadvantages, including interruption in the continuity of work and the frequent loss of the services of workers who have gained some experience. It was recommended that this system should largely be replaced by the support of nutrition research centres of a more permanent nature at selected places where suitable laboratories exist, together with a nucleus of experienced workers and competent direction. This will ensure continuity in work and provide opportunity for the training of skilled nutrition workers as well as for the development of regional nutritional organisations. It is to be expected that such centres, in accordance with their development, their contributions to the study of local nutrition problems and the functions they may perform for the Provincial Government will also receive support from local sources. The financing of nutritional research along such lines should not, however, preclude the support of temporary schemes in other centres which are likely to produce fruitful results.

10. VILLAGE PROJECT SCHEMES.

The Committee considered a proposal put forward by the Agricultural Commissioner with the Government of India about the collaboration of nutrition workers in " village project schemes ". The Imperial Council of Agricultural Research has defined the Village Project System as follows :—

" A project is a planned system of land use involving the simultaneous or coordinated application of a variety of measures of proved value. A project is carried out on the land of a farmer *by* himself (with the help or guidance of Government technicians) as a means of permanently improving his land, his cultivation practices, his production and his income. A project is most likely to be successful both technically and economically when it is carried out on a group of contiguous holdings or, best of all, a whole village ".

The objectives of Village Project Schemes were explained to the Committee by the Vice-Chairman, Imperial Council of Agricultural Research, and the Agricultural Commissioner with the Government of India. They suggested that nutrition experts could co-operate in village project schemes, e.g. by indicating the amounts of various foods required to supply the inhabitants of a village with a balanced diet, thus providing an objective towards which agricultural improvements could be directed. It was further pointed out that if the necessary foods were produced it might be difficult to ensure that the people actually eat these—that each member of the community got his necessary share.

The Committee was in favour of collaboration, since one of its most important objectives is the development of contact between nutrition and agriculture with a view to influencing agricultural policy by the findings of nutrition research. Various means by which nutrition workers could participate in village project schemes were discussed and the matter was left for further discussion at the next meeting of the Committee after consideration of ways and means in the interval by the Agricultural Commissioner and the Chairman of the Nutrition Advisory Committee.

11. Employment of provincial nutrition officers.

The conditions arising out of the present food crisis have further emphasised the importance of having trained nutrition officers attached to public health departments in all Provinces and the major Indian States. The lack of such officers in many provinces has seriously weakened the organisation needed for dealing with the urgent problems which have arisen in connection with the food situation. The Committee strongly urged that Provinces should arrange to have nutrition officers trained and employed under the scheme recommended by the I. R. F. A. in 1937. Whole-time nutrition officers in public health departments should have the status of Assistant Directors of Public Health.

12. The food situation.

The Committee in its discussions of the food situation distinguished between Bengal, where very severe famine exists, and other parts of India where the situation is less serious but requires careful watching. As regards Bengal and the food situation in general, the following recommendation was made.

"*Imports of Foods.* India has never been a self-sufficient country as regards food supplies in the sense that the population is adequately or abundantly fed. The war has led to an increase in food requirements and simultaneously to a cutting off of normal food imports. Very severe famine exists in Bengal and there is serious shortage in other parts of the country. In view of these facts, the Committee recommends that immediate steps should be taken to obtain food, by import if necessary, in sufficient quantity to tide over the present emergency and reduce loss of life in the Bengal famine. At the same time, a strong co-ordinated effort must be made to produce more food in India".

The conditions in Bengal were vividly described to the Committee by the members from that Province. Thousands of destitute people in a famished state, often suffering from other conditions superimposed on starvation, are to be found wandering from their homes and thronging the streets of Calcutta. One of the first necessities is to discover the best methods of treating those who are admitted to hospitals dangerously ill and often in a state of collapse. The Committee recommended that a grant of Rs. 12,000/- approximately should be given by the Indian Research Fund Association to the All-India Institute of Hygiene to organise clinical research into this problem immediately. The enquiry should be based on the All-India Institute of Hygiene and Public Health. Any results obtained should at once be made available to medical officers and hospitals throughout Bengal, through the Surgeon General. It was emphasised that in evolving suitable methods of resuscitation and treatment the conditions under which patients are hospitalised, and the medical and nursing attention available, should be borne in mind. Methods must be simple and as easily and widely applicable as possible. The Surgeon General stated that such an enquiry would be of the greatest value.

The Committee also emphasised the necessity that expert nutritional advice should be taken in deciding the composition of 'gruel' and other food mixtures issued to destitutes in feeding centres, camps and hospitals. The 'gruel' at present given in food distribution centres in Calcutta supplies less than 800 calories per adult daily and is not altogether suitable in composition. The Surgeon General, Bengal, stated that he would welcome and make use of expert advice in such matters.

Provinces other than Bengal.—In July 1943, Dr. Aykroyd drew up a 'Note on the study of existing food situation and its effect on the state of nutrition and

health of the population' which was circulated to Surgeons General, Inspectors General of Civil Hospitals and Directors of Public Health. Its object was to suggest methods by which a watch could be kept on the food situation from the standpoint of nutrition and public health, so that by collecting and collating accurate information governmental policy and public opinion could be influenced. The Committee considered this note and the methods of approach suggested in it, at its meeting on October 1st. The same subject was discussed at a joint meeting of the Nutrition Advisory Committee and Administrative Medical Officers and Directors of Public Health on the following day.

The Committee recommended that the problem should be approached by (a) diet surveys and (b) investigation of the state of nutrition of sample groups. Diet surveys should be of a simple type which would serve to give a rough and ready idea of the diet in good and bad areas. For example, it would be of value to ascertain the *per capita* intake of cereals in sample groups of families. This could be done by workers with a limited training. The areas to be investigated and the number of type of families to be selected for the enquiry could be decided by local authorities in consultation with nutrition workers.

With regard to methods of investigating state of nutrition, the Committee considered the report of a Conference on Methods and Procedures for the Nutrition Surveys of Population Groups which was held in Atlantic city, U.S.A., in October 1941. Under present circumstances the rapid collection of information is necessary and many of the methods described in the report are not suitable for this purpose and would require more expert workers than are available. The Committee drew from the Report a list of symptoms and physical signs indicative of malnutrition which could be rapidly recorded by relatively inexperienced clinical workers. The list of symptoms and physical signs is printed as Appendix I to this report. The Committee suggested that the work could be carried out in consultation with the Administrative Medical Officers and Directors of Public Health. It was considered that this work should as far as possible be carried out by the staff of the Medical and Public Health Departments and of any existing school medical service. It was represented that in some Provinces difficulties would be experienced owing to depleted staff, but it was considered that a solution of this difficulty might be found in the employment of senior medical students. In fact, it was suggested that such work might be regarded as part of the hygiene training in medical curriculum.

Special emphasis is placed on two points : loss of weight and impaired efficiency for work. Data were presented by the Director of Public Health, Madras, showing that the average weight of children in Madras Corporation School was lower in the year beginning April 1st, 1943, than the average for the preceding 5 years. Loss of weight in the circumstances is a useful index of state of nutrition. Impaired efficiency for work is also an early sign of deterioration in nutrition. Information about any reduction in the output of work by groups of manual workers in rural areas could, for example, be obtained from P. W. D. officers in charge of labour groups. In urban areas statistics of output of work in factories would afford a clue.

Shortage of food, involving not only a low intake of calories, but also insufficiency of protein, vitamins, etc. will inevitably influence vital statistics and the incidence of disease. The value of the study of vital statistics as an index of the effect of the food situation on health was discussed at the meeting attended by Administrative Medical Officers and Directors of Public Health. The methods suggested in Dr. Aykroyd's memorandum include study of the

general death rate, the mortality in various age groups (notably children and old people) and the mortality and morbidity figures for various diseases which usually became more prevalent in times of food shortage and famine. From the discussion it clearly emerged that, except perhaps in certain areas (e.g. the Madras Presidency and various Municipalities) no immediate information of value on the effect of the food situation could be obtained in this way owing to the long delay in the transmission and collation of the necessary vital statistical data by the inadequate statistical organisations under Directors of Public Health. Under these circumstances Directors of Public Health should take steps to institute special enquiries into morbidity and mortality in deficit areas and in areas where for other reasons a deterioration in food supplies and public health is suspected. Regarding morbidity statistics from dispensaries and hospitals, while the view was expressed that these would fail to indicate at a very early stage a deterioration in health, nevertheless it was agreed that they should be closely studied by Administrative Medical Officers and Directors of Public Health.

13. Rationing.

The Committee discussed rationing schemes and made the following recommendation :—

" Cereals form the great bulk of most Indian diets, intake of other foods on the part of the majority of the population being small. The food the rationing of which is of primary importance in connection with the food situation is cereals. In order, however, to ensure an equitable distribution of ingredients other than cereals which form part of an adequate diet, rationing schemes should also include the rationing of pulses, oils and fats, sugar, etc.

The cereal rations allowed under existing rationing schemes are low and the poorer classes cannot obtain a sufficiency of other foods to cover their calorie requirements. The fact should be realised in adjusting ration scales. No doubt the amount of the ration must depend on the supplies of food immediately available. But if rationing is considered as part of a general policy of food production and distribution, then some reasonably satisfactory level of intake should be adopted as an objective. One pound of cereal per capita, (including adults and children) may be regarded as a suitable minimal figure. This assumes a normal age distribution of the population ; it would not, for example, be suitable for groups largely composed of adult male labourers or manual workers. It assumes that the country is faced with some shortage of cereal grains and that the objective to be aimed at is to carry the population through the shortage without too serious an impairment of health.

In fixing the ration of items other than cereals in any given area, advice from nutrition experts, if possible experts with local knowledge, should be sought.

Special provision must be made for covering the needs of manual workers. One method of dealing with this question is the development of canteen feeding in industrial establishments.

The rations allowed to children under existing schemes are small. Children's rations could be supplemented by the development of school feeding schemes, to which part of the available food supplies could be diverted. This would be of great value from the standpoint of public health, since growing children are particularly likely to suffer in health in times of food shortage. Special consideration should be given to the needs of expectant and nursing

mothers and as far as possible milk supplies should be reserved for them and for children."

The Committee strongly recommended that immediate action should be taken along these lines, where rationing schemes are contemplated.

14. Substitutes for rice.

The Committee considered a letter dated September 11th, 1943, from the Controller of Rationing, Bombay, to Dr. W. R. Aykroyd, in which the Controller stated that the Bombay Government have now decided to undertake publicity to encourage the consumption of various cereals, particularly bajra (Pennisetum typhoideum) in place of rice, in view of the over-all shortage of rice in the country and in consideration of the position in Bombay city. He asked for a "publicity slogan" about the nutrition value of bajra which could be used in educating the public The Committee's conclusion on this letter was as follows :—

"As far as possible, in organising rationing schemes, people should be supplied with the cereal to which they are accustomed. A sudden change over from one type of cereal to another may cause indigestion, but healthy persons should in time be able to adapt themselves to a change in diet. There is no objection, from the standpoint of nutrition to the partial substitution of rice by wheat, bajra or other cereals, and there is no scientific basis for the idea that bajra generates heat in the body. If bajra is substituted for rice in the diet of rice-eaters, methods of cooking bajra in a palatable and digestible form must be taught to the people."

APPENDIX I

Certain symptoms and signs suggestive of malnutrition. An abbreviated list taken from " Nutrition Survey of Population Groups. Report of a conference on Methods and Procedures."

U. S. Public Health Reports. Vol. 57. February 6, 1942. No. 6.

A. Symptoms and signs suggestive of early deficiency states in infants and children.

Symptoms.	Physical signs.
1. Lack of appetite.	1. Lack of sub-skin fat.
2. Failure to gain steadily in weight.	2. Poor muscle tone.
3. Aversion to normal play.	3. Rough skin ; phrynoderma.
4. Chronic diarrhoea.	4. Sores at angles of mouth.
5. Pain on sitting and standing.	5. Red tongue.
6. Backwardness in school.	6. Square head, wrists enlarged, rib beading.
7. Repeated respiratory infections.	7. Vincent's angina, thrush.
8. Photophobia.	8. Serious dental abnormalities.
9. Lacrimation.	9. Corneal and conjunctival changes.

B. Symptoms and signs suggestive of early deficiency states in adolescents and adults which may exist in absence of under-weight or other evidence of undernourishment.

Symptoms.	Physical signs.
1. Lack of appetite.	1. Angular stomatitis.
2. Lassitude and chronic fatigue.	2. Glossitis.
3. Loss of weight.	3. Stomatitis.
4. Diminished output of work.	4. Spongy bleeding gums.
5. Loss of strength.	5. Muscle tenderness, extremities.
6. Chronic diarrhoea.	6. Bilateral symmetrical dermatitis.
7. Sore bleeding gums.	7. Dermatitis, facial butterfly, Casal's necklace, perineal, scortal, vulval.
	8. Anaemia.
	9. Rough skin : phrynoderma.

REPORT OF THE TENTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN NEW DELHI ON THE 13th and 14th DECEMBER, 1943.

COMPOSITION OF THE COMMITTEE.

Lieut.-Colonel E. Cotter, C.I.E., I.M.S., Public Health Commissioner with the Government of India (*Chairman*).

Professor S. P. Agharkar, Professor of Botany and Head of the Botany Department, University College of Science, Calcutta.

Dr. K. P. Basu, Lecturer in Chemistry and Incharge of the Biochemical Laboratory, University of Dacca, Dacca.

Khan Bahadur Dr. A. H. Butt, Director of Public Health, Punjab, Lahore.

Dr. B. C. Guha, Ghosh Professor of Applied Chemistry, University College of Science and Technology, Calcutta.

Dr. V. N. Patwardhan, Asst. Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Dr. G. Sankaran, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Mr. F. Ware, C.I.E., Animal Husbandry Commissioner with the Government of India.

Dr. W. R. Aykroyd, C.B.E., Director, Nutrition Research Laboratories, I.R.F.A., Coonoor (*Secretary*).

The following were present by invitation :—

Dr. Bashir Ahmad, University Professor of Organic Chemistry, University of the Punjab, Lahore.

Dr. C. Chandrasekaran, Assistant Professor of Vital Statistics, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. M. B. Daver, Nutrition Officer, Medical and Public Health Department, Hyderabad-Deccan.

Dr. K. Mitra, M.B.E., Officer-in-Charge Nutrition Scheme, Public Health Laboratories, Bankipore, Patna.

Dr. Mohd. Musa Khan, Nutrition Officer, Public Health Department, Punjab, Lahore.

Dr. K. C. Sen, Officer-in-Charge, Animal Nutrition Section, Imperial Veterinary Research Institute, Izatnagar.

Lieut.-Colonel A. M. Thomson, R.A.M.C., Assistant Director (Nutrition), Medical Directorate, New Delhi.

1. Statistical control of diet surveys.

The Committee considered a detailed memorandum on methods of carrying out dietary surveys and their statistical control, prepared by Drs. Aykroyd, Mitra and Sankaran, in accordance with a recommendation in the December 1942 report. The memorandum was generally approved of. In one of its sections a period of 10 days for diet surveys was recommended, with repeated surveys of similar length at various seasons of the year. Certain members considered that a survey period of 7 days is adequate ; if the investigation is extended much beyond this length the families concerned readily become tired of the survey and less ready to co-operate. It was suggested that the length of period necessary to obtain a satisfactory picture of dietary habits depends on daily variations in food intake. Various members of the Committee experienced in diet survey work expressed the view that these are normally small. Study of the degree of variation would throw light on the time of survey necessary for statistically valid results. It was proposed that detailed daily records of food intake obtained in surveys should be sent to Dr. Chandrasekaran, Assistant Professor of Vital Statistics, All-India Institute of Hygiene and Public Health, Calcutta, for investigation with the above object in view.

2. Distribution of Health Bulletins Nos. 23, 28 & 30.

Health Bulletin No. 23, " The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets ". A third edition of 15,000 copies was printed in 1941. In December, 1943, 5,385 remained unsold, some 4,300 having been disposed of during the year. Of the Urdu edition of 5,000 copies, printed in 1942, 4,218 copies remain unsold. Some 200 copies have been disposed of during 1943.

Health Bulletin No. 28, " Rice ". First printed in 1940. 817 copies remain, about 100 having been sold during the year.

Health Bulletin No. 30, " Food and Diet (written for children) ", 5,000 copies were printed in 1942. In December, 1943, only one copy remains unsold.

3. Village project schemes.

The co-operation of nutrition workers in " village project schemes " sponsored by the Imperial Council of Agricultural Research was discussed at the October 1943 meeting of the Committee, at which the Vice-Chairman of the Imperial Council of Agricultural Research and the Agricultural Commissioner with the Government of India were present. The Chairman of the Nutrition Advisory Committee reported that the matter had been further discussed with the Vice-Chairman and the Agricultural Commissioner. Village project schemes are located in the United Provinces, the Central Provinces, Bombay and Travancore. In none of these are there full time nutrition officers attached to the Public Health Department who could give advice about village project schemes from the nutritional angle. It was proposed that agricultural officers in charge of village project schemes should receive a two months course of training in nutrition in Coonoor, subject to it being found possible to arrange with the provinces concerned to relieve the workers in question for this period. While the Imperial Council of Agricultural Research assists in financing the scheme, the staff employed are the servants of the Provincial Governments. It is desirable that the course should be held at a season of the year at which agricultural activities are slack. It was suggested that workers on schemes in certain provinces might be trained in the All-India Institute of Hygiene and Public Health, Calcutta, rather than in the Nutrition Research Laboratories, Coonoor.

4. NUTRITION UNITS.

At its meeting held on the 1st and the 2nd October, 1943, the scheme submitted by Dr. B. C. Guha to largely replace the system of supporting temporary research schemes at different centres by the creation of Nutrition Research Units of a more permanent nature was adopted and the present nutrition research centres under Dr. Guha, Dr. Basu, Dr. Ahmad and Dr. Patwardhan at Calcutta, Dacca, Lahore and Bombay respectively were mentioned in this connection. This question was taken up in detail at the present meeting and the Committee recommended the creation of Nutrition Research Units in Dacca and Bombay, to start with, into which the existing enquiries at these centres were recommended to be absorbed. It was considered that an annual grant of Rs. 12,000/- would be suitable to start with for each of these centres. It was also decided that the question of forming similar Nutrition Research Units under Dr. Guha and Dr. Ahmad in Calcutta and Lahore respectively, which had been envisaged in the previous meeting of the Nutrition Advisory Committee should be brought up for consideration in course of the next year.

5. THE CLINICAL TREATMENT OF FAMINE DESTITUTES IN BENGAL.

At its meeting in October 1943, the Committee recommended that a grant of Rs. 12,000/- approximately should be given to the All-India Institute of Hygiene and Public Health, Calcutta, to organize clinical research into methods of treating sick starving destitutes. This sum was immediately made available and the investigation started without delay. On December 1st, a report entitled "Treatment and management of starving sick destitutes" was issued from the All-India Institute of Hygiene and Public Health, Calcutta. Two hundred copies were given to the military authorities concerned with medical famine relief and 600 copies to the Medical Department of the Government of Bengal.

The Committee expressed its appreciation of the important wo k rap dly and efficiently carried out by the clinical research unit in Calcutta and recommended that the enquiry should be continued during 1944-45 if necessary.

6. NUTRITION WORK IN PROVINCES AND STATES.

Work carried out during the year included the following :

Punjab : Reports on nutrition work carried out by the Punjab Public Health Department in 1943 were presented. A report entitled "An enquiry into diets, state of nutrition and factors associated therewith, in relation to health in the Lyallpur district, Punjab" was printed during the year. This contains valuable and interesting data and suggests a number of practical measures for improving nutrition and health in the district concerned. Diet surveys were carried out on iron foundry workers in Batala (an industrial area in Gurdaspur district) and a diet survey was also made of 314 boarders in the hostels of Khalsa College, Amritsar.

Progress was made in the experiment on the prevention of goitre by iodised salt in Kangra, the first experiment of the kind in India. The public health problem presented by goitre in certain parts of India is an extensive and important one.

A careful watch was kept on the effect of the food situation on health and nutrition. The data collected showed that there was no acute distress amounting to scarcity or famine in any part of the province. Diets consumed by the

poorer classes in towns were, however, found to be unsatisfactory. These contain enough calories yielded by cereals and pulses to satisfy hunger, but they are almost devoid of protective foods. It was observed that the average intake of cereals per capita daily in rural areas amounted to 37 ounces and in ubran areas to 17 ounces, the provincial average for both urban and rural areas being 32 ounces. These facts are of considerable importance in connection with rationing schemes in the Punjab.

A study was made of the vital index-ratio of births to deaths—in towns with a population of 30,000 and upwards. It was found that the trend of the ratio was upward throughout most of the province, indicating that there was no general deterioration in public health as a result of the food situation. In the Hissar district the position was less satisfactory, the ratio being below 100. Investigations had also been carried out on mortality in infants and old people, groups in the population which are particularly likely to suffer in times of food shortage.

Bihar : Further dietary surveys of Muslim families were carried out in the Saran district. In the course of this survey the relation between income and diet was studied. Nutritional propaganda has been issued by the Public Health Laboratories.

In order to study the effect of the food situation on diet, 458 families included in a previous survey during 1938-40 were subjected to further investigations in September-November, 1943. It was found that intake of all kinds of foods had diminished, the fall being particularly marked in the case of cereals. Estimated reduction in cereal intake was of the order of 30 to 45 per cent. Families were observed in which consumption of cereals did not exceed 5 oz. per consumption unit daily. Maize and millet were largely consumed in the district in which the families are located. The food situation has improved with the arrival of the harvest.

Some 3,000 children in the same area were examined. The percentage incidence of malnutrition, as judged by general clinical assessment, was found to be about 40 per cent, as compared with 25-30 per cent in previous survey.

Hyderabad : During the year surveys were carried out on 250 families in the Asifabad and Karimnagar districts. The diets were found to be in general ill-balanced. Intake of leafy vegetables was low. Vitamin A deficiency was found to be common.

The value of "mahwa" yeast as a source of B vitamins was investigated in collaboration with the Coonoor Laboratories. Since this kind of yeast was found to be satisfactory as regards its vitamin content, its use in hospitals in the State has been recommended. Following a recommendation made by the Medical and Public Health Department, a Government order was passed restricting the polishing of rice. The Nutrition Section of the Public Health Department has carried out education and propaganda work.

A rapid survey of 3 districts in which surveys had previously been made was carried out in November 1943. This showed that there has been no deterioration in total food intake. The income of the families has increased since the previous surveys. The diet is, however, very deficient in quality and there has been some increase in deficiency diseases in certain areas.

The Committee expressed its appreciation of the nutrition work carried out in the Punjab, Bihar and Hyderabad and considered that the efforts being made to keep a watch on the effect of the food situation on diet and health are

particularly commendable. It hoped that other provinces and states would follow the good example set by the public health departments concerned.

7. School feeding scheme in Cochin.

The Committee took note of the scheme inaugurated in November 1943 by the Dewan of Cochin for the feeding of school children in the State, with reference to the recommendation made in its report of October 1943 that children's rations should be supplemented by the development of school-feeding schemes, to which part of the available food supplies could be diverted. It is proposed to supply free midday meals to all children in Cochin attending Primary Schools, who at present number about 180,000. The Government, subject to the approval of the Legislative Council, will meet one-third of the cost, the remaining two-thirds being met by public subscription. The children will be given foods other than rice, at a cost of not more than an anna per head daily. The grain supplied will be mainly wheat, but some tapioca and bajra will also be available. In the initial stages powdered and condensed milk from South Africa will be used if this can be obtained in time.

8. Preparation of a report for the Health Survey and Development Committee.

The Committee was asked to prepare a report on nutrition for the Health Survey and Development Committee. Nutrition *research* has already been considered by the Nutrition Advisory Committee ; what is now required is a report on nutrition in its practical and public health aspects. The subject may be considered under the following heads :

(a) The existence and extent of under and malnutrition in India as shown by agricultural production data and surveys of diet and state of nutrition.

(b) Deficiency diseases.

(c) Education and propaganda.

(d) Nutrition departments in Provinces and States and public health nutrition work generally.

(e) Plans for improving nutrition. Under this head the following questions may be considered :

  (i) Co-ordinated measures for improving nutrition by suitable development of agriculture, animal husbandry and fisheries. (Irrigation and forestry may be briefly considered under this head).

  (ii) The creation of permanent food departments, Central, Provincial and States, concerned with co-ordinating measures for the production and distribution of food.

  (iii) The population problem and nutrition.

  (iv) The possible role of synthetic vitamins and substances rich in vitamin (yeast, shark liver oil) in raising the nutritional level of the population.

It was suggested that members of the Committee should prepare *brief* notes on some or all of the above heads and send them to the Secretary in Coonoor not later than January 31st, 1944. The Secretary will then attempt to prepare a composite report which can be considered by the Committee at a meeting in March, 1944. It is to be observed that the report, while it should be comprehensive and cover the important aspects of the subject, cannot go into the various questions in detail. It should aim at giving a broad outline of the problem of nutrition and indicate briefly the most important measures which should be followed in attempting its solution.

9. EXISTING AND PROPOSED ENQUIRIES.

The Committee recommended the continuation of the following enquiries:—

(i) Nutrition researches under Dr. W. R. Aykroyd at the I.R.F.A. Nutrition Research Laboratories, Coonoor and the additional nutrition enquiry.

(ii) Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(iii) Studies in human nutrition with Indian diets under Dr. K. P. Basu, at Dacca University, Dacca.

(iv) Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

Lieut.-Colonel Thomson, Assistant Director in Nutrition, G. H. Q., India, offered his collaboration in this enquiry, by, if possible, obtaining facilities for Mr. Datta to carry out investigations on Indian soldiers in hospitals in Bombay.

(v) Enquiry on the minimum and optimum requirements of dietary fat under Drs. S. P. Niyogi and V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(vi) Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

It was recommended that Mr. M. N. Rudra should consult Dr. B. C. Guha about his programme for the coming year.

(vii) Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the University Chemical Laboratories, Lahore.

(viii) Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipur, Patna.

(ix) Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Dr. B. N. Banerjee at the Indian Institute of Science, Bangalore.

(x) Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

(xi) The Committee considered the report presented by the Soya-Bean Sub-Committee which is attached to this report. It noted that considerable progress has been made in investigating the nutritive value of soya-bean in comparison with that of other pulses. It

recommended that the further investigations suggested by the Sub-Committee should be continued for another year, when a full report of the work carried out should be published. Two of the soya-bean enquiries will be absorbed into the nutrition units referred to above.

The following enquiries will terminate in 1944 :—

(i) Studies on the metabolism of vitamin A and carotene under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

Work on this subject will be taken over by the All-India Institute of Hygiene and Public Health, Calcutta.

(ii) Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

The following new enquiries were recommended :—

(i) Enquiry into the various constituents of the vitamin B complex under Dr. K. P. Basu at Dacca University, Dacca.

(ii) Enquiry into the estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.

(iii) Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

(iv) Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(v) Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras.

The following enquiries were not recommended :—

(i) Enquiry into (i) a new hormone 'artosterone' prepared from artostenone occurring in Jack fruit and (ii) vitamin D from sterols and their physiological significance in nutrition under Dr. M.C. Nath at Dacca University, Dacca.

(ii) Epidemic dropsy enquiry under Dr. S. N. Sarkar at Dacca University, Dacca.

(iii) Studies on the body surface area of Indians under Dr. D. M. Telang at the Grant Medical College, Bombay.

The following enquiries were not considered by the Committee, since they related to applications for Medical Research Fellowships and were not in the form of proposals usually put up before the Committee :—

(i) Enquiry on beriberi, pellagra, riboflavin deficiency and post puerperal peripheral neuritis under Dr. T. K. Raman at the Andhra Medical College, Vizagapatam.

(ii) Enquiry into the etiology of hepatic cirrhosis in Bengal with special reference to the role of dietetic deficiency under Dr. J. C. Banerjea at the Indian Institute for Medical Research, Calcutta and the Medical College Hospital, Calcutta.

(iii) Enquiry on clinical dermatological conditions associated with faulty diet under Dr. K. Gupta at the Medical College Hospital, Calcutta.

REPORT OF THE SOYA-BEAN SUB-COMMITTEE.

December 13th, 1943.

The Sub-Committee met first in December, 1941, when it drew up a programme of research, the object of which was to determine the nutritive value of soya-bean in comparison with that of various common Indian pulses. During the current year a considerable amount of further work has been carried out in four laboratories under Drs. Ahmad, Aykroyd, K. P. Basu and Patwardhan. The results obtained from the various laboratories, in certain lines of work recommended by the Sub-Committee, show a close measure of agreement. The Sub-Committee is therefore in a position to report these as representing a definite contribution to the problem.

(1) *Experiments on rats* : The nutritive value of soya-bean and other pulses was investigated as follows :

(a) The biological value of the proteins of the various pulses was tested by the balance-sheet method.

(b) The same by the growth method.

(c) The effect of soya-bean and other pulses in supplementing a " poor rice diet " was investigated. The nature of the poor rice diet was previously agreed on by the Sub-Committee. The other pulses tested were Bengal gram (*Cicer Arietinum*), green gram (*Phaseolus radiatus*) and red gram (*Cajanus indicus*).

The results of all 3 methods of investigation may be briefly summarized. The biological value of soya-bean protein, tested by the balance-sheet and the growth methods, was found to be of the same order as that of the proteins of the other pulses. In the majority of experiments, Bengal gram showed a slight but definite superiority to the other pulses. The effect of soya-bean in supplementing the poor rice diet was also of the same order as that of the other pulses. Here again a slight superiority in the case of Bengal gram was evident.

All this work was carried out on uniform samples of the various pulses supplied to the different workers. In this way the possibility of obtaining divergent results by the use of different varieties was eliminated.

(2) *Human metabolism experiments* : The biological value of soya-bean protein was also tested by metabolism experiments on human beings. Here again soya-bean and the other pulses gave results of the same order. One variety of soya-bean from Lyallpur gave a higher value than that given by a Kashmir variety.

(3) *Feeding experiment on school children* : An experiment lasting 6 months, was carried out in Lahore. The important comparison in this case was between soya-bean and dhal arhar, which were given to the children in similar amounts and in the same way as supplements to their ordinary diet. Statistical analysis of the results showed no signficant difference in the weight increments of the two groups during the experimental period. This was the case in all age groups.

The Sub-Committee is of the opinion that the metabolism and growth experiments carried out with rats are reasonably complete. A considerable bulk of data from the four Laboratories is available. The feeding experiments on children have also given a definite answer. These results should be published as a contribution to the subject under consideration.

FUTURE WORK.

(1) The Sub-Committee considered that the experiments in which the pulses were given as supplements to a poor rice diet cannot be regarded as complete and should not be published at this stage. It is puzzling that soya-bean, with its high protein content, should not be in some degree superior to other pulses as a supplement. In order to throw light on this problem, experiments should be carried out to investigate the importance of other limiting factors in the poor rice diet, notably deficiency of calcium, vitamin A, and the vitamin B complex. If the diet is supplemented by these various factors, it is possible that soya-bean may show some superiority to other pulses.

(2) Further work should also be carried out (a) on the effect of heating on the nutritive value of soya-bean and (b) on the comparative value of different varieties of soya-bean, and (c) metabolism experiments on human beings.

The Sub-Committee feels that when this work has been carried out with particular reference to the investigation on the limiting factors on the poor rice diet, there will be a reasonable hope of giving (in 1944) a final answer to the question at issue, viz. whether the nutritive value of soya-bean, in comparison with that of common Indian pulses, is such as to justify, from the standpoint of human nutrition, the encouragement of the production and consumption of soya-bean on a wide scale in India.

It is expected that most of the soya-bean work will be carried on in the permanent nutrition units recommended by the Nutrition Advisory Committee in its October 1943 report, so that the situation has altered with regard to the special financing of soya-bean investigations.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on 13th December, 1943, the following members being present :

Lieut.-Col. S. S. Sokhey (*Chairman*).
Dr. R. Adiseshan.
Lieut.-Col. L. A. P. Anderson.
Dr. V. R. Khanolkar.
Dr. C. G. Pandit.
Major-General Sir John Taylor (*Secretary*).

The reports of the enquiries in progress during the year were considered.

(1) PLAGUE ENQUIRY UNDER THE DIRECTOR, HAFFKINE INSTITUTE, BOMBAY.

(a) *Plague vaccine.* The Committee note with special interest the results of investigations on the protective value of vaccine of different types including broth and agar vaccines grown at different temperatures and killed and preserved in different ways. The work has shown that a vaccine consisting of a suspension of a three days culture of a virulent strain grown at 37°C with a content of 1000 million organisms per cubic centimetre gives a degree of protection equal to that produced by the Haffkine vaccine or other type of broth vaccine. It has also been shown that the toxicity of such an agar grown vaccine is about one-fifth of that of the broth vaccine also tested. As determined by a precipitin test the content of envelope antigen in the agar vaccine is similar to that of the broth vaccines. In the case of all vaccines it was found that toxicity was considerably reduced by the addition of 0.1% formalin.

Phenyl mercuric nitrate was found to be a satisfactory preservative for the vaccine.

(b) *Treatment of plague with sulphadiazine.* As cases are only now becoming available for trials of the theraputic action of sulphadiazine a sufficient number have not yet been done to provide figures for comparison with those obtained by the use of other preparations.

Proposals for 1944-45. The Director, Haffkine Institute proposes to continue the studies on the relative value of broth and agar-grown vaccines and in particular to determining the keeping properties of agar-grown vaccine. It is intended to carry out field trials of agar vaccine with the assistance of the Director of Public Health, Madras who has just completed a statistical study on the value of the Haffkine vaccine.

The Committee are agreed that it is important to carry out the field trials as agar vaccines may present considerable advantages in use and in manufacture. It is suggested that comparisons should be made between the value of agar-grown vaccine prepared from virulent strains of *P. Pestis* and standard stable strains of the protective avirulent type.

It is proposed to continue the trials of sulphadiazine in treatment of plague at Poona in 1944-45 in event of a sufficient number of cases not being available during the present financial year. The Committee recommend that for the purpose of these investigations the grant of Rs. 11,240/- asked for should be allotted viz.

Salaries	.. Rs. 5,240/-
Contingencies	.. Rs. 1,000/-
Field trials	.. Rs. 5,000/-

In the event of the work on treatment with sulphadiazine being completed in the present financial year the allotment of Rs. 5,000/- for field trials will not be required.

2. PLAGUE ENQUIRY AT HOSUR UNDER THE DIRECTOR OF PUBLIC HEALTH, MADRAS.

The Committee note the work that has been done on the natural history of plague including the studies on rat and flea populations in the area and the results of control by fumigation.

The studies on sylvatic plague and of the influence of microclimates on endemic prevalence are considered to be of value. The results of studies on the pulicidal value of pyrethrum suspension are considered to be inconclusive and this work requires extension with the use of watery or other types of suspensions as well as oily suspensions.

PROPOSALS FOR 1944-45.

The Director of Public Health Madras, proposes to continue the work in the Hosur Taluk during the coming year and to study.—

(a) Rodent fleas and their prevalence on different species of wild rodents and squirrels in relation to sylvatic plague and persistence of infection.

(b) Micro-climatic conditions in relation to recrudescence.

(c) Rat-proof construction and the re-modelling of existing buildings to render them rat-proof.

(d) Cyanogas fumigation to confirm the results of the Cumbum Valley investigation.

The trials of sulphathiazole in treatment of plague will be carried out in the Coimbatore area as better facilities exist there than in Hosur.

The Committee recommended that support be given to the enquiry on the above lines by a grant of Rs. 2,500/- towards the total estimated cost of Rs. 14,600. The Director of Public Health, Madras, has represented that with rising costs for staff etc. further funds may be required and has requested consideration of raising the grant to Rs. 3,000/-.

(3) The Director of Public Health, Madras, put before the Committee the results of a statistical examination of the results of antiplague inoculation with the Haffkine vaccine during an epidemic in Coimbatore town. The following figures are extracted from the Report :—

	Inoculated.	Uninoculated.
Average weekly population ..	93,616	82,512
Attacks ..	239	774
Attack rate per mille (all) ..	2.34	9.38
Attack rate per mille (in persons attacked not less than 7 days after inoculation) ..	1.60	..
Deaths ..	90	452
Case mortality ..	37.65	58.40

The protection against attack indicated by these figures is approximately four-fold in the case of all inoculated and six-fold in the case of persons inoculated 7 days before onset and at a period which would permit of development of immunity. The figures are much of the same order as those based on the analysis of the results obtained over a large series of inoculation campaigns.

INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

A. (1). *Volume XXX* (1942).—*The Journal.*—Vol. XXX coincided with the calendar year and the usual 4 quarterly numbers were published. The average size for a number was 164 pages of literary and tabular matter and 3 plates, representing a decrease of 69 pages quarterly as compared with the previous volume.

(2). *The Memoirs.*—One *Memoir* (No. 34—March 1942)—'A comparative Nutritional Survey of various Indian Communities' by D. C. Wilson and E. M. Widdowson—was published with this volume.

(3). *The net cost of Vol. XXX* (inclusive of *Memoir* No. 34) was Rs. 6,747.

B. (1) *Volume XXXI* (1943)—*The Journal*—Owing to the reduction in the amount of material which had been experienced it was decided to publish only two numbers annually during the War. Also, in the interest of economy and on account of the shortage of paper a new style of printing with narrower margins was adopted which resulted in the utilization of 25 per cent more space per page. Further reduction in the use of paper by running on of articles could not be made possible on account of technical difficulties in the production of reprints. The two numbers of Vol. XXXI appeared in May and October and the average size was 122 pages with 2 plates as compared with 160 pages and 4 plates in the volume of the previous year.

(2). *The Memoirs.*—No *Memoir* was published with this volume.

C. *Distribution.*—

Indian subscribers	.. ..	117	= 211
Foreign subscribers	.. ..	94	
Exchange list	.. ..	182	= 304 (less 75 suspended owing to War=229)
Free list	.. ..	122	
Voucher copies to advertisers, file, regn:, make-up, police, specimen, propaganda, etc.	.. ..	40 approx.	
Total	.. ..	555 or 440	
Balance left with Publishers for sale	..	145 or 260	(actual now)
Total copies printed	.. ..	700	

The Exchange list has been increased by 3 over last year by addition of new addresses. There has been a rise of 5 in Indian and 8 in Foreign subscribers as compared with last year. In addition, 75 Foreign addresses on the distribution lists have been suspended owing to the War. There has thus been a total increase of 16 in the distribution as compared with last year. A large number of publications are obtained in exchange for the I. J. M. R. for the I.R.F.A. Library, though some from enemy countries have been suspended

D. *Distribution lists.* These were last revised in July 1938 and a new corrected alphabetical list showing exchanges has been printed and copies are available from the office of the Secretary for distribution to workers under the I.R.F.A.

E. *Number of copies printed.*—From July 1940 it has been decided to print only 700 copies instead of 900 as this number is considered sufficient, according to former experience, to meet all demands. Volumes I to VIII are out of print.

F. *The cost of printing the Journal.*—The following statement shows the cost of production during the last five years :—

COST OF PRINTING THE JOURNAL.

Number.	XXV-XXVI 1938–39.	XXVI-XXVII 1939–40.	XXVII-XXVIII-XXIX, 1940–41.	XXIX-XXX 1941–42.	XXX 1942.	
April ..	2,415	3,276	5,137	2,052	2,949	700 copies
July ..	4,744	4,212	3,932	3,531	1,650	
October ..	3,801	4,611	4,717	3,413	2,413	
January ..	3,652	3,640	3,571	2,531	Not published	
Total ..	14,612	15,739	17,357	11,527	7,012	
Average pages	251	260	317	214	158	

RECOVERIES.

April ..	2,965	1,628	1,452	1,325	1,034
July ..	1,429	1,628	1,451	1,325	946
October ..	1,048	838	963	837	1,518
January ..	1,048	838	963	837	..
Total ..	5,490	4,932	4,629	4,324	3,498

G. *Net cost of the Journal and Memoirs.*—The following statement shows the net cost of the production of the *Journal* and *Memoirs* during the last five years :—

JOURNAL.

April ..	—549	1,648	3,685	727	1,915
July ..	4,316	2,584	2,481	2,206	704
October ..	2,752	3,773	3,754	2,576	895
January ..	2,603	2,802	2,608	1,694	..
Total ..	9,122	10,807	12,528	7,203	3,514

MEMOIRS.

	No. 30	No. 31	..	No. 33	..
	2,849	1,764	..	1,565	..
	..	No. 32	..	No. 34	..
		1,632		1,627	
Totals ..	2,849	3,386	..	3,192	..
Grand Total	11,971	14,193	12,528	10,395	3,514

For convenience of accounting all receipts are credited to the *Journal* account and the figures given for the *Memoirs* are the actual cost of production.

The net cost during 1942 after deduction of receipts was Rs. 3,514. All Publishers' bills for 1942 were submitted in time to make payments within the financial year.

H. *Financial position for the year* 1943-44.—The budget allotment for the year was Rs. 7,000 for the cost of 2 half-yearly numbers of the *Journal* and Rs. 2,000 for any *Memoir* which might be published. The following are the actual and estimated expenditures :—

Vol. XXXI. No. 1, May, 1943..	.. Rs.	1,796 (Actual)
Vol. XXXI. No. 2, October, 1943	.. Rs.	3,500 (estimated)
No. *Memoir* published ..	..	Nil.
Total	.. Rs.	5,296

The saving anticipated is Rs. 3,704.

J. *General.*—Publication of the *Journal* has been continued on the lines laid down at the Conference of 1932 and the services of the members of the Editorial Board have been made full use of by referring to them all papers dealing with the subjects to which they have been nominated. There is at present no member for Helminthology.

Although a revised type of printing has been adopted for the duration of the War the *Journal* has been well turned out by the printers and its appearance is satisfactory.

In Vol. XXXI (May and October 1943) out of 37 articles published 15 were on nutrition, 6 on bacteriology, 3 on biochemistry, 4 on pharmacology and 5 on haematology.

K. *Budget estimate for* 1944-45.—It is intended to continue publishing only two numbers of the *Journal* during 1944 which will be produced in May and October. The following is the estimated cost of publication during the year for 700 copies of 2 numbers and 1 *Memoir* which may possibly be received :—

2 half-yearly issues of the *Journal* at Rs. 3,500 ..	Rs. 7,000
1 *Memoir*	Rs. 2,000
Total ..	Rs. 9,000

The annual subscription has been reduced from Rs. 16 to Rs. 8, while the cost of individual single numbers remains as before.

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1943.

A. *Indian Journal of Medical Research and Indian Medical Research Memoirs.*

1. BASU, K. P., and GHOSH, D.—Availability of Calcium in Lady's Finger (*Hibiscus esculentus*), Cabbage (*Brassica Oleracea capitata*), Drumstick (*Moringa Oleifera*) and Amaranth Tender (*Amaranthus gangeticus*) Part I. Availability of Calcium in Vegetables determined by Experiments on growing Rats (Vol. 31, No. 1).
2. BASU, K. P., and GHOSH, D.—Availability of Calcium in Lady's Finger (*Hibiscus esculentus*), Cabbage (*Brassica Oleracea capitata*), Drumstick (*Moringa Oleifera*) and Amaranthus Tender (*Amaranthus gangeticus*). Part II. Availability of Calcium in Vegetables determined by Metabolism Experiments on a Human Adult (Vol. 31, No. 1).
3. CHOPRA, R. N., GUPTA, J. C., BOSE, B. C., and CHOPRA, I. C.—Hypnotic Effect of *Rauwolfia serpentina*: The Principle underlying this Action, its Probable Nature (Vol. 31, No. 1).
4. DHARMENDRA.—Immunological Skin Tests in Leprosy. Part IV. The Isolation of Three different Protein Fractions from *Mycobacterium Leprea* (Vol. 31, No. 2).
5. DHARMENDRA.—Immunological Skin Tests in Leprosy. Part V. A Bacillary Antigen Standardized by Weight (Vol. 31, No. 2).
6. DHARMENDRA, and BOSE, R.—Bactericidal Action *in vitro* of Sulphanilamide and Sulphapyridine on *Mycobacterium leprea muris* (Vol. 31, No. 2).
7. GREVAL, S. D. S., BHATTACHARJI, J. N., and DAS, B. C.—Determination of Blood Groups from Stains (Vol. 31, No. 2).
8. GREVAL, S. D. S., BHATTACHARJEE, J. N., and ROY CHOWDHURY, A. B.—On Isoheamolysis : Reports on Two Isolysins and Associated Considerations (Vol. 31, No. 2).
9. GREWAL, K. S., and KOCHHAR, B.D.—Luffa acutangula: The Chemical and Pharmacological Investigation of Luffa Seeds (Vol. 31, No. 1).
10. GUPTA, J.C., and KAHALI, B.S.—Comparative Pharmacology of the Total Alkaloids of *R. serpentina Benth.* obtained from Bengal, Bihar and Dehra Dun (Vol. 31, No. 2).
11. KAMLA BHAGVAT.—Combined estimation of Thiamin and Nicotonic Acid in Foodstuffs by Chemical Methods (Vol. 31, No. 2).
12. KAMLA BHAGVAT and SARMA, P. S.—Accumulation of Pyruvic Acid in Rice Moth Larvea (*Corcyra cephalonica* St.) fed on a Vitamin B_1-deficient Diet (Vol. 31, No. 2).
13. KIRWAN, E. O'G., SEN, K., and BOSE, N.—Nutrition and its Bearing on Preventable Blindness and Eye Disease in Bengal (Vol. 31, No. 1).
14. MARSHALL DAY, C. D. and SHOURIE, K. L.—The Effect of Vitamin C on Gingival and Periodontal Disease in Indian Children (Vol. 31, No. 2).
15. MITRA, K., and MITTRA, H. C.—Determination of the Biological Value of Proteins from Red Ants (*Oecophylla smaragdina*) by the Balance-Sheet Method (Vol. 31, No. 1).

16. NAPIER, L. E., and SEN GUPTA, P. C.—Studies in Haemolysis : with 'Histological Notes' by N.V. Bhaduri and 'Statistical Analysis' by C. Chandra Sekar (Vol. 31, No. 1).
17. NIYOGI, S. P., PATWARDHAN, V. N., and ACHARYA, B. N.—Studies in Fish-Liver Oils. Part I. The Biological Assay of Vitamins A and D in Ghol (*Scicana miles*) and Mushi (*Scoliodon sorrokowah*) Liver Oils (Vol. 31, No. 1).
18. NIYOGI, S. P., PATWARDHAN, V. N., and ACHARYA, B. N.—Studies in Fish-Liver Oils. Part II. The Seasonal Variation in the Yield and Vitamin A Content of some Fish-Liver Oils (Vol. 31, No. 1).
19. PANJA, G., and GHOSH, S. K.—The Action of Dyes on Vibrios (Vol. 31, No. 1).
20. SARMA, P. S.—The Effect of 'Exercise' on the Pyruvic Acid Content of Normal and Vitamin B_1-deficient Rice-Moth Larvea (*Corcyra cephalonica* St.) (Vol. 31, No. 2).
21. SARMA, P. S.—Riboflavin and Pyridoxin (Vitamin B_6) as Growth-Promoting Factors for Rice-Moth Larvea (*Corcyra cephalonica* St.) Vol. 31, No. 2).
22. SEKHON, NIRANJAN SINGH.—The Effect of Storage on the Carotene content of Dehydrated Vegetables (Vol. 31, No. 2).

B. *Journal of the Malaria Institute of India.*

1. BASU, B. C.—Laboratory studies on the infectivity of Anopheles annularis. (Vol. V, No. 1).
2. COVELL, G.—Prophylaxis and treatment of malaria in war. (Vol. V, No. 2).
3. COVELL, G., and JASWANT SINGH.—Antimalaria operations in Delhi, Part IV. (Vol. V, No. 1).
4. JASWANT SINGH and JACOB, V. P.—Abstract. Malaria in Ahmedabad. (Vol. V, No. 1).
5. KNOWLES, R., and BASU, B. C.—Laboratory studies on the infectivity of *Anopheles stephensi*. (Vol. V, No. 1).

C. *List of papers published by workers under the I. R. F. A. in Journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

AYKROYD, W. R.—Vitamin B_1 Deficiency in Infants (Journal of the Association of Medical Women in India, 1943, Vol. XXXI, No. 2, p. 41).

AYKROYD, W. R.—The United Nations Conference on Food and Agriculture (Current Science, 1943, Vol. XII, No. 8, p. 217).

AYKROYD, W.R.—The Nutrition of School-Children (The South Indian Teacher, Dec. 1942).

BHAGVAT, K.—Combined Determination of Thiamin and Nicotinic Acid in Foodstuffs by Chemical Methods (Current Science, 1943, Vol. XII, No. 4, p. 116).

BASU, K.P., and KARKUN, J.N.—"Glucose dehydrogenase from germinated seeds of green and black grams (phaseolus radiatus and phaseolus mungo, Linn ". (Jour, Ind. Chem. Soc., Vol. XX; No. 7).

BASU, K. P., and KARKUN, J. N.—"Succinic acid dehydrogenase from cucumber seeds". (Jour. Ind. Chem. Soc., Vol. XX; No. 8).

BRAGANCA, B. M., GHOSH, N. C., and SAHA, K. C.—Vitamin A and D contents of certain fish-liver Oils. (Annls. Biochem. Exptl. Med., Vol. II, No. 2)

COCHRANE, R. G.—The Leprosy Campaign in the Madras Presidency with special reference to Prevention. (The Journal of the Christian Medical Association of India, Burma & Ceylon, July, 1943).

COCHRANE, R. G.—The study of Family Susceptibility in relation to the Epidemiology of Leprosy. (Leprosy in India, Vol. XV, No. 3, July 1943).

COCHRANE, R. G.—Some aspects of Tuberculous Infection in Saidapet. (Indian Medical Gazette, Vol. LXXVIII, No. 4, (April 1943).

COCHRANE, R. G.—Epidemiology, Pathology and Diagnosis of Child Leprosy—being the Elizabeth Matthai Endowment Lectures of the University of Madras (1943) : (in print).

COVELL, G.—Malaria and War. *J. United Ser. Institution Ind.*, Vol. 73, No. 312.

DAS GUPTA, C. R., and SEN GUPTA, P. C.—Agranulocytosis in kala-azar. (*I.M.G.*, Vol. 78, No. 1).

DAS GUPTA, C. R.—Indian-made liver extracts in the treatment of macrocytic anaemia. (*I.M.G.*, Vol. 78, No. 1).

DAS GUPTA, C. R.—Transmission of malaria through transfusion of blood. (*I.M.G.*, Vol. 78, No. 8).

DHARMENDRA and N. MUKHERJI (1943).—Seasonal variation in the activity of lesions of the neuromacular cases of leprosy. Lep. in India, Vol. XV, No. 1

DHARMENDRA and S. S. JAIKARIA (1943).—Studies of the lepromin test (10) Results of the test with various antigens in non-contacts. Lep. in India. Vol. XV, No. 2.

DHARMENDRA (1943). What the people should know and do about leprosy. Lep. in India, Vol. XV, No. 2.

DHARMENDRA and LOWE, J. (1943). Studies of the lepromin test (11) A summary of the work done in the present study. Lep. in India, Vol. XV, No. 3).

DHARMENDRA and N. SEN (1943).—Epidemiological and clinical studies of leprosy in the Bankura District of Bengal. Lep. in India, Vol. XV, No. 4.)

GHOSH, B. N.—Studies on the effect of certain experimentally induced physiological disturbances on the bio-synthesis of ascorbic acid by the rat. (Ann. Biochem. Expt. Med., Vol. III, No. I.)

GUPTA, J. C., DEV, A., and KAHALI, B. S.—Preliminary observation on the use of Rauwolfia serpentina Benth in the treatment of mental disorders. *I.M.G.*, Sept. 1943.

HARDIKAR, S. W., and Rao, V. G.—Ascites in Hyderabad (Dn). (Jour. Ind. Med. Assoc., Vol. XIII, No. I, October, 1943).

NAPIER, L. E., DAS GUPTA, C. R., and SEN GUPTA, P. C.—Acute macrocytic leukaemia in Indians. (*I.M.G.*, Vol. 78, No. 1).

PANSE, T. B. and PARANJPE, A. S—A study of "Carpasemine isolated from Carica Papaya seeds. (Proc. Ind. Acad. Sc., 1943, Vol. XVIII, Sec. A, p. 140).

RUDRA, M. N.—Ascorbic Acid Content of Recently Harvested Cereals and Legumes. (Nature, Vol. 152, No. 3846, July 17, p. 78.)

RAO, S. SUNDAR,—The result of amputation of a limb for filarial lymphangitis and elephantiasis. (*I.M.G.* Vol. LXXVIII, No. 2).

SOKHEY, S. S. and HABBU, M. K.—Optimum and Limiting Temperatures for the growth of the Plague Bacillus in Broth, (Jour. of Bacteriology, Vol. 46, No. 1, July 1943, pp. 25-32).

SOKHEY, S. S. and HABBU, M. K.—Optimum and Limiting Hydrogen-ion concentrations for the growth of the Plague Bacillus in Broth, (Journal of Bacteriology, Vol. 46, No. 1, July 1943, pp. 33-37).

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 14th and 15th December, 1943.

	Enquiries.	Budget allotments Rs.
1.	Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta	4,480
2.	Malaria Institute of India, Research Section, Delhi ..	37,358
3.	Southern India Branch of the Research Section of the Malaria Institute of India, Coonoor	12,544
4.	Nutrition Research Laboratories, I.R.F.A., Coonoor ..	82,771
5.	Additional nutrition enquiry, (a) Assistant Director, Nutrition Research Laboratories, I. R. F. A., Coonoor, (b) three field workers and (c) enquiry on beri-beri in Coconada. (*The cost to be met from the balance of Rs.* 30,000/- *annual grant sanctioned by the Governing Body over a period of five years, out of the special ten lakhs grant made by Government for anti-malaria and nutrition work.*)	24,652
6.	Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	5,848
7.	Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	2,164
8.	Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna	1,893
9.	Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmed at the University Chemical Laboratories, Lahore ..	2,534
10.	Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipore, Patna	2,233
11.	Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore	2,390
12.	Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. N. Khankolar at the Tata Memorial Hospital, Bombay	2,312
13.	Enquiries into (i) tests of the biological value of soya-bean (ii) growth tests with soya-bean (iii) human experiments with soya-bean under Dr. Bashir Ahmed at the Institute of Chemistry, Punjab University, Lahore	1,920
14.	Nutrition Research Unit under Dr. K. P. Basu at the Dacca University, Dacca	12,000

Enquiries.	Budget allotments. Rs.
15. Nutrition Research Unit under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	12,000
16. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta	7,612
17. Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras	2,190
18. Plague researches under the Director, Haffkine Institute, Parel, Bombay	11,240
19. Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta	10,546
20. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra at the Drug Research Laboratories, Jammu, Kashmir ..	4,447
21. Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay	3,469
22. Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	5,188
23. Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta	6,296
24. Enquiry entitled Studies in tests of physical fitness in Indians under Dr. D. M Telang at the Grant Medical College, Bombay	2,013
25. Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta	5,520
26. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	4,997
27. Kala-azar enquiry under the Director, Pasteur Institute, Shillong	12,952
28. Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay	3,969
29. Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta ..	6,600
30. Enquiry into the occurrence of ascites in Hyderabad State under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan	992
31. Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	2,980
32. Enquiry on typhus under Dr. S. R. Savoor at the Haffkine Institute, Bombay	11,532
33. Statistical enquiry into the methods of forecasting epidemic malaria under Khan Bahadur Dr. M. Yacob, Assistant Director of Public Health, Punjab, and Mr. Satya Swaroop, Statistical Officer, Lahore	1,532

	Enquiries.	Budget allotments. Rs.
34.	Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmed at the Punjab University Institute of Chemistry, Lahore	2,374
35.	Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay, Rs. 8,488. The Board asked that the Director, Haffkine Institute should submit for the approval of the Chairman and Secretary a revised budget excluding provision for the purchase and feeding of rats.	
36.	Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	3,424
37.	Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras	2,632
38.	Enquiry into vaccine lymph under the Director of Public Health, Bombay	300
39.	Enquiry into Naga sore under Col. H. E. Shortt, C.I.E., I.M.S., I. G. C. H., Assam, Shillong	8,360
40.	Enquiry into the fraction of liver most suitable for the treatment of tropical macrocytic anaemia under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	3,380
	GRANTS.	
(i)	Administration charges	37,301
(ii)	Contribution towards the cost of two professorships at the School of Tropical Medicine, Calcutta	27,387
(iii)	Contribution towards the cost of plague enquiry in the Salem District under the Government of Madras	3,000
(iv)	Contribution to the Imperial Institute of Entomology, London (£ 100/-)	1,350
(v)	Contribution to the Bureau of Hygiene and Tropical Diseases, London (£ 150/-)	2,025
(vi)	Contribution towards the cost of the Asst. Public Health Commissioner with the Government of India and the Statistical Bureau in the office of the Public Health Commissioner with the Government of India	6,650
(vii)	Four research fellowships of the value of Rs. 150/- p.m. each ..	7,350
(viii)	Indian Journal of Medical Research and Memoirs	
	(a) Publication charges of the Journal 7,000	
	(b) Publication charges of the Memoirs .. 2,000	
	(c) Editor's office 4,530	13,530

Grants.	Budget allotments. Rs.
(ix) I. R. F. A. Library at the Central Research Institute, Kasauli ..	1,880
(x) Pay (including special pay, overseas pay and allowances) and contributions towards passage, leave salary and pension or contributory provident fund of the officers of the Medical Research Department under the Association	1,50,000
(xi) Travelling allowance and certain contingent expenses for Medical Research Department Officers employed under the I. R. F. A. and attached to Central or Provincial laboratories ..	2,000
(xii) Pay and travelling allowance of the two workers in the Junior Research Cadre	13,950
(xiii) Clerical assistance at the School of Tropical Medicine, Calcutta	3,606
(xiv) Clerical assistance to the Director, All-India Institute of Hygiene and Public Health, Calcutta	1,368
(xv) Travelling allowance of subordinate I.R.F.A. workers on transfer from one post to another	1,500
(xvi) I. R. F. A. contribution and interest towards the Contributory Provident Fund	13,000
(xvii) Miscellaneous unforeseen expenditure	15,000
(xviii) Provision for imprest advance for enquiries	7,790

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1944.

LIBRARY
OF THE
BOMBAY
BRANCH
OF THE
ROYAL ASIATIC SOCIETY

Issued under the authority of the Governing Body,
Indian Research Fund Association,
New Delhi,
India.

Price per copy One Rupee.

Obtainable from the Secretary, Governing Body, Indian
Research Fund Association, Secretariat,
NEW DELHI (India).

With the compliments of
Lieut.-Colonel C. A. Bozman, I.M.S.,
Secretary, Governing Body and
Scientific Advisory Board, Indian
Research Fund Association.

NEW DELHI
INDIA.

LIBRARY OF THE ROYAL BOMBAY BRANCH OF THE ASIATIC SOCIETY

CONTENTS.

	Page.
I. Composition of the Board	i
II. Composition of Advisory Committees	ii
III. Meetings held during the year	iii
IV. Researches carried out during the year	1—112

CHOLERA.

Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta	1—4
Enquiry on the statistical evaluation of anti-cholera inoculation in the Madras Presidency under the Director, King Institute, Guindy, Madras and the Director of Public Health, Madras	4—21

MALARIA.

Malaria Institute of India (Research Section), Delhi and Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi	22—25
Statistical enquiry into the methods of forecasting epidemic malaria under Khan Bahadur Dr. M. Yacob, Assistant Director of Public Health, Punjab, and Mr. Satya Swaroop, Statistical Officer, Public Health Department, Punjab, Lahore	25—26

NUTRITION

Nutrition researches under Dr. W. R. Aykroyd at the I.R.F.A. Nutrition Research Laboratories, Coonoor	27—30
Ascorbic acid enquiry under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	30—31
Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	32—35
Enquiry on the role of manganese on the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna	35—36
Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore ..	36—37
Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipur, Patna	37—38

Page.

Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore .. .. 38—39

Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay .. .. 39—46

Enquiry into (1) tests of the biological value of soya-bean protein (2) growth tests with soya bean (3) human experiments with soya bean under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore 46

Nutrition research unit under Dr. K. P. Basu at the Dacca University, Dacca .. .. 46—50

Nutrition research unit under Professor S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay .. 50—53

Enquiry into methods of treating sick starving destitutes in Calcutta based on the All-India Institute of Hygiene and Public Health, Calcutta .. .. 53—57

Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore 57

Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay .. .. 57—58

Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta .. .. .. 59—60

Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras .. .. .. 61—63

LEPROSY.

Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta .. .. 64—67

Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras .. 68—76

PLAGUE.

Plague researches under the Director, Haffkine Institute, Bombay .. .. 77—79

Plague enquiry in the Salem District under the Director of Public Health, Madras .. .. 79—80

PHARMACOLOGY.

Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta .. .. .. 81—82

Page.

Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S. at the Drug Research Laboratory, Jammu, Kashmir .. .. 82—85

Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay 85

OTHER RESEARCHES.

Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta .. .. .. 86—87

Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta .. .. 87—89

Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay .. .. 89—90

Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta .. .. 90—93

Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras .. .. 93—94

Kala-azar enquiry under the Director, Pasteur Institute, Shillong 94—96

Enquiry on anerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay .. .. 96—97

Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta .. 97—99

Enquiry into the occurrence of ascites in Hyderabad State under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan 100

Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta .. .. 101—102

Enquiry on typhus under Dr. S. R. Savoor at the Haffkine Institute, Bombay .. .. 102—104

Enquiry into vaccine lymph under the Director of Public Health, Bombay .. .. 104—109

Enquiry into Naga sore under the Inspector-General of Civil Hospitals, Assam, Shillong .. .. .. 109—110

Enquiry into the fraction of liver most suitable for the treatment of tropical macrocytic anaemia under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta .. .. 110—111

Enquiry on schistosomiasis in the army under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta .. .. 111—112

V. Reports of Advisory Committees on

Cholera .. .. 113—115

Malaria .. .. 116—119

Nutrition .. .. 120—158

Page.

Plague .. .. 159—160

Anti-smallpox vaccination in British India .. .. 161—177

VI. Indian Journal of Medical Research and Indian Medical Research Memoirs .. .. 178—181

VII. Miscellaneous papers issued under the auspices of the Indian Research Fund Association .. .. .. 181—183

VIII. Programme of researches and miscellaneous grants recommended for financial year 1945-46. 184—188

The Scientific Advisory Board consisted of the following :-

Chairman :

Major-General J. B. Hance, C.I.E., O.B.E., K.H.S., I.M.S., Director-General, Indian Medical Service (1st January to 10th August and 24th December to 31st December, 1944).

Major-General W. C. Paton, M.C., K.H.P., I.M.S., Offg. Director-General, Indian Medical Service (11th August to 23rd December, 1944).

Members :

Colonel E. Cotter, C.I.E., I.M.S., Public Health Commissioner with the Government of India, New Delhi. *Secretary.*

Major-General Sir John Taylor, C.I.E., D.S.O., I.M.S., Director, Central Research Institute, Kasauli (1st January to 13th February, 1944).

Lieut.-Colonel H. W. Mulligan, I.M.S., Director, Central Research Institute, Kasauli (14th February to 31st December, 1944).

Rai Bahadur Dr. B. M. Das Gupta, Director, School of Tropical Medicine, Calcutta.

Lieut.-Colonel S. S. Sokhey, I.M.S., Director, Haffkine Institute, Parel, Bombay.

Dr. C. G. Pandit, O.B.E., M.B.B.S., Ph.D., D.P.H., D.T.M., Director, King Institute, Guindy, Madras (1st January to 6th May and 18th June to 8th August, 1944).

Dr. K. V. Venkatraman, M.D., Offg. Director, King Institute, Guindy, Madras (7th May to 17th June and 9th August to 31st December, 1944).

Brigadier G. Covell, C.I.E., I.M.S., Director, Malaria Institute of India, Delhi (1st January to 6th August and 20th September to 31st December, 1944).

Major Jaswant Singh, I.M.S., Offg. Director, Malaria Institute of India, Delhi (7th August to 19th September, 1944).

Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S., Director, Drug Research Laboratory, Jammu, Kashmir.

Dr. A. N. Goyle, M.D., Ph.D., Professor of Pathology, King Edward Medical College, Lahore.

Dr. V. N. Patwardhan, M.Sc., Ph.D., Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Co-opted member :

Dr. W. R. Aykroyd, C.B.E., M.D., Director, Nutrition Research Laboratories, Coonoor (for the subject of nutrition).

Composition of Advisory Committees for the year 1945.

1. *Cholera.*

Lieut.-Colonel H. W. MULLIGAN (*Chairman*).
Colonel E. COTTER.
Dr. B. B. DIKSHIT.
Dr. C. G. PANDIT.
Dr. S. R. PANDIT.
Rao Bahadur Dr. K. C. K. E. RAJA.
Lieut.-Colonel S. S. SOKHEY.
Major M. L. AHUJA.
Dr. K. V. VENKATRAMAN. } (*Joint Secretaries*).

2. *Malaria.*

Brigadier G. COVELL. (*Chairman*).
Brigadier M. K. AFRIDI.
Dr. B. ANANTHASWAMY RAO.
Rai Bahadur Dr. A. C. BANERJEA.
Brevet-Colonel Sir RAM NATH CHPORA.
Rai Bahadur Dr. B. M. DAS GUPTA.
Lieut.-Colonel H. W. MULLIGAN.
Dr. D. K. VISWANATHAN.
Major JASWANT SINGH. (*Secretary*).

3. *Nutrition.*

Colonel E. COTTER. (*Chairman*).
Professor S. P. AGHARKAR.
Dr. K. P. BASU.
Khan Bahadur Dr. A. H. BUTT.
Sir THEODORE GREGORY.
Dr. B. C. GUHA.
Dr. V. N. PATWARDHAN.
Dr. G. SANKARAN.
Mr. J. SARGENT.
Mr. H. R. STEWART.
Major G. WILLIAMSON.
Dr. W. R. AYKROYD. (*Secretary*).

4. *Plague.*

Dr. K. V. VENKATRAMAN. (*Chairman*).
Lieut.-Colonel L. A. P. ANDERSON.
Dr. A. N. GOYLE.
Dr. V. R. KHANOLKAR.
Dr. S. R. PANDIT.
Lieut.-Colonel S. S. SOKHEY. (*Secretary*).

The Board held two meetings during the year, the first on the 27th November and the second on the 28th and 29th November, 1944.

At its meeting on the 27th November, 1944, the Board confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers, and also decided on the co-option of experts for its meeting on the 28th and 29th November, 1944. On these dates the Board considered in detail the programme of research for the year 1945-46. The programme of researches recommended by the Board is given on pages 184-188 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1944.

The researches, which were carried out during the year under report, and which were recommended by the Board at its meeting on the 14th and 15th December, 1943, were approved by the Governing Body of the Association at their meeting held in the Imperial Secretariat, New Delhi, on the 18th March, 1944.

CHOLERA.

1. Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta.

TREATMENT OF CHOLERA CASES WITH SULPHAGUANIDINE.

It has been reported before that sulphaguanidine was administered to 250 cases of cholera along with intravenous injections of saline at the Campbell Hospital with promising results. As almost all these cases were admitted more or less in a state of collapse intravenous saline had to be given. In order to ascertain the specific effect of the drug in cholera before the onset of collapse when saline is not indicated, it was decided to try the drug in early cases in the field.

During 1943-44 there was an outbreak of cholera at Joynagar in the district of 24-Parganas and at Bajitpur in Mymensingh district, and it was possible to treat 97 cases in these areas. Of these 53 had sulphaguanidine and the remaining 44 cases were treated with other drugs. This latter group of 44 cases was considered as controls. None of the 97 cases were given saline. Stools from 10 patients were examined and *V. cholerae* (Ogawa) was isolated from 8 patients. There was no doubt as to the diagnosis of cholera in the remaining cases as judged by the clinical symptoms exhibited by them, even without carrying out bacteriological examinations. The results of the treatment are given in the following table :

	Sulphaguanidine treated cases.	Controls.
Total No. of cases	53	44
Recovered	42 or 79 %	11 or 25 %
Died	9 or 17 %	33 or 75 %
Unknown	2 or 4 %	..

IN VIVO AND IN VITRO EXPERIMENTS WITH SULPHAGUANIDINE.

Tests were carried out for vibriocidal and vibriostatic properties.

(a) *Vibriostatic—V. cholerae* were grown in 9 c.c. of nutrient broth tubes for 4 hours. The drug in different dilutions in 1 c.c. amounts was then added to the tubes and incubated. These were subcultured on bile-salt agar media

at 1, 2, 4, 24 and 48 hours of incubation. There was no growth on the bile-salt plates thus indicating the presence of vibriostatic property. In some of the lower dilutions, subculture in liquid medium showed no growth indicating thereby the bactericidal property.

(b) *Vibriocidal*—Various dilutions of vibrio cholerae were added to different tubes of broth containing the same amount of sulphaguanidine in each and incubated for 24 hours. There was no apparent growth of vibrios in any of the broth tubes. 5 c.c. from each of these tubes were added to 100 c.c. nutrient broth flasks and incubated. No growth appeared in any of the flasks after 24 hours, but after 48 hours, growth of vibrio cholerae appeared in some of the flasks containing low dilutions of *V. cholerae* and no growth could be seen in any of the flasks containing high dilutions of the organisms. This shows that sulphaguanidine has both bactericidal and bacteriostatic effect *in vitro*, although the bactericidal effect is poor.

Combined test. Agar plates with different dilutions of drugs were prepared by adding different dilutions of solutions in 10 c.c. distilled water to 10 c.c. melted agar (double strength) and pouring the mixture into sterile plates aseptically. Then 24 hours old broth culture of *V. cholerae* were plated out on them and incubated. The plates containing high dilutions of sulphaguanidine gave good growth of vibrios, thus neither bactericidal or bacteriostatic effect was apparent. When the concentration of the drug in the plates was increased, the vibrio colonies on the plates appeared small, fine and few in number, indicating bacteriostatic effect. Finally when the concentration of the drug was still more increased there was no growth on these plates and subculture from the plates into liquid medium also showed no growth, indicating thereby bactericidal effect.

Sulphasuxidine in cholera cases.

One hundred and eighteen cases of clinically diagnosed cholera were treated with sulphasuxidine. Most of the cases were admitted in bad condition. Alternate cases were treated as controls. Intravenous saline therapy was necessary in both the groups and transfusions were done as often as necessary. The death rate among each of the sulphasuxidine treated and control cases was 10%.

The dosage used for an adult weighing 50 Kg. was 25 grm of sulphasuxidine per day (divided into 6 doses) for 2 days and 12.5 grm a day divided into 6 doses for another day, (as recommended by Drs. Poth, Firor and Feirer in a letter to the D.G., I.M.S., dated 4.12.41).

Dosage :—

1st dose		5 grm or 10 tablets.
2nd dose after 4 hours	=	4 grm or 8 tablets.
3rd „ „ „	=	do.
4th „ „ „	=	do.
5th „ „ „	=	do.
6th „ „ „	=	do.
6 doses in 24 hours	=	25 grm or 50 tablets.

The same dose was given on the 2nd day of treatment and sulphasuxidine was continued with half the usual dosage on the 3rd day.

So far no difference could be seen in improvement in signs and symptoms of cholera in the sulphasuxidine treated and control groups of cases.

INVESTIGATION OF CHOLERA IN TIRHUT DIVISION, NORTH BIHAR.

An investigation was undertaken into the virulence of cholera in Tirhut Division, North Bihar, and it was carried out on the following lines :

1. Visits to cholera hospitals and infected villages.
2. Collection of stools of suspected cases of cholera and plating them out immediately on bile-salt media.
3. Incubation of the plates, and study of the morphology and serology (slide agglutination) in the field laboratory.
4. Sub-cultures of *V. cholerae* isolated were sent to the School for biochemical and detailed serological (Dreyer's method) study, and virulence test in animals.
5. Study of the presence of agglutinins in samples of blood from convalescents.
6. Miscellaneous studies—Conditions of general health and malnutrition of the public, prevalence of other diseases, sources of propagation of the disease, etc.

N.B. Bacteriological and phage study of samples of water could not be done as the wells were generally disinfected.

The investigations into the conditions of the 13 villages in different districts where the epidemic was still going on were carried out. Stools of acute cases were examined by cultural method. *V. cholerae* (Ogawa subtype) was isolated from 70% of the cases suffering from clinical symptoms of cholera in 10 out of 13 villages, leaving no doubt that the epidemic was really one of cholera. In the other 3 villages, *viz.* Mirpurjoara, Bhagwanbazar and Purandarpur, there were 13 cases in all out of which 5 were bacteriologically negative. Stools of 8 cases that died were not available for examination. The convalescent case in Mirpurjoara was examined on the 10th day of the disease. In Bhagwanbazar, there was only one case suffering from acute diarrhoea. Three cases were sent from Purandarpur to the hospital at Bettia. These were found to be suffering from acute dysentery.

Virulence test of strains. 6 strains of *V. cholerae* isolated in North Bihar were tested for virulence in guinea-pigs and it was found that these were not more virulent than the strains isolated from sporadic cases in Calcutta during the same period.

In order to get further proof about the etiological factor of the epidemic in villages where actual cases were not available, blood was collected from 7 convalescent cases (10—15 days after the attack) to study the development of agglutinins in their blood. 5 samples had agglutinins in titre of 1/40. This suggests that the epidemic may be one of cholera.

Other factors besides *V. cholerae* responsible for the virulence of the disease are :—

(1) The people of those localities who were used to eating rice had to take recourse to sweet potatoes, maize, etc., as their staple food on account of the

shortage of their normal foodstuff. This is responsible for malnutrition amongst the affected population. This was evident from the local information.

(2) Malaria, hookworm, etc., are also very common in those localities. Such diseases are also responsible for the low vitality of the people.

(3) The disease suddenly spread to such a wide extent that it was not possible to send adequate medical relief and to take necessary prophylactic measures to check the epidemic in such a wide area effectively. The disease was brought under control wherever proper measures were adopted.

(4) From the records of deaths due to cholera in previous years, it appears that there were severe epidemics in 1918 and 1930. This year a severe epidemic has occurred again. Whether such periodicity in the occurrence of the epidemic is due to normal periodic behaviour should be examined.

The sanitary condition of the villages was very poor. Flies were found to be breeding in large numbers and that perhaps was one of the causes of the spread of this epidemic.

2. Enquiry on the statistical evaluation of anti-cholera inoculation in the Madras Presidency under the Director, King institute, Guindy, Madras, and the Director of Public Health, Madras.

1. Apart from Russell's survey carried out in 1925-26 on limited statistical material on the comparative value of anti-cholera inoculation and oral administration of Bili-Vaccine in individual protection against cholera, it does not appear that surveys based on sufficiently large amount of statistical material have been carried out to assess the value of anti-cholera vaccine in the prevention and control of cholera. Apart from this, the anti-cholera vaccine which is used at present in this Province contains both OGAWA AND INABA strains of the cholera vibrio ; it is probably different from the vaccine used in Russell's survey and no statistical data is available about its efficacy. For these reasons, it was considered that the very large number of anti-cholera inoculations done during the wide-spread and severe epidemics of cholera in the Province of Madras in 1942 and 1943 afforded an exceptionally good opportunity of an investigation to assess the value of anti-cholera inoculations in the conferment of personal immunity and of the influence of mass immunity in the prevention and control of cholera epidemics. Investigation of this subject was suggested to the Scientific Advisory Board of the Indian Research Fund Association in October, 1943 and the proposal was readily accepted. Approval of the Madras Government was obtained and with the financial assistance from the Government and a subvention from the Indian Research Fund Association the present investigation was undertaken.

2. As a basic knowledge of the geography of the Province, the important facts relative to the epidemiology of cholera in the Province, and of the system of Public Health administration in vogue with special reference to notification and prevention and control of epidemics, would be useful, these subjects are dealt with briefly in the preliminary paragraphs of the report.

3. The Province of Madras which is one of the major Provinces in British India, occupies the whole southern portion of the Peninsula. Exclusive of Indian States, it has an area of about 126,000 square miles and a population of nearly 50 millions. It has on the East Coast on the Bay of Bengal a coast line of about 1000 miles and on the West on the Arabian Sea, a coast line of about 450 miles. A plateau occupies the central area of the Province. On either side are the Eastern and Western Ghats. The Province thus lies in the following natural regions :

(a) West Coast region.

(b) The Northern Circars Region comprising the Northern Part of the East Coast, lies between the Eastern Ghats and the Bay of Bengal.

(c) The Carnatic or Tamil Region—This includes the Southern part of the East Coast.

(d) The Deccan Plateau comprises the inland Ceded districts. The districts comprised in the groups (a), (b) and (c) get their rainfall from the South-West Monsoon during the months June to August, whilst those in (c) get the rainfall principally from the North-East Monsoon during the months of October to December and to some extent from the South-West Monsoon. The average annual rainfall of the Province is about 50 inches of which 26 inches are derived from the South-West monsoon, 16" from the North-East monsoon and the balance 8 inches from local summer showers.

The Northern Circars Region is irrigated by two large rivers, viz., the Godavari and Kistna and the Carnatic Region by the Cauvery and the Tambaraparani. The areas under irrigation in these two areas are generally known as the Delta Areas and it is believed that some of these are endemic for cholera.

4. The Public Health administration is more or less parallel to the system of Revenue Administration which is briefly as follows :

The Executive Chief for each of the 25 districts in the Presidency is the Collector. Every district is composed of a number of Taluks—8 to 12 in a district. The Taluk Revenue Official is the Tahsildar. Each of the taluks comprises several villages—ranging anywhere from 50 to 200. Besides the main portion, every village contains several suburbs which are designated in this Province as " hamlets ". These hamlets may contain either more or less self-contained units of population or specific population groups, e.g., weavers, farmers, artisans, unskilled labourers, etc., who have communal lives of their own. The village Revenue official is the headman.

Every district contains a certain number of municipalities ranging from one to six. There are altogether 82 Municipal towns in the Presidency ; the civic administration of Municipal towns and rural areas is governed by the Municipal Act and Local Boards Act respectively.

5. As regards Public Health establishment, the great majority of the 82 municipalities have Health Officers who are Government officers and subordinate staffs who are municipal employees ; where there are no Municipal Health Officers, the Executive Authorities are in charge of routine Public Health administration and they seek the advice of the District Health Officer in technical matters. For the rural areas which form the bulk of each district both as regards size and population, there are a Health Officer and an Assistant Health Officer.

Generally, for each taluk (called a range) there is a Health Inspector who is a qualified Sanitary Inspector. The Health Officers, Assistant Health Officers and Health Inspectors belong to the Provincial Service. In every district there are in addition to the above staff, Vaccinators, Licence Inspectors and others in inferior services who are servants of Local Bodies. Many of these subordinates are qualified Sanitary Inspectors and can be diverted for Public Health work other than their primary duties. The whole of the public health subordinate staff in each unit—Municipal or Rural—is under the administrative control of the Health Officer.

It may also be mentioned that under the Madras Public Health Act, Municipal Authorities are statutorily bound to provide for Public Health administration a minimum of 30 per cent of their total revenues and District Boards a minimum of 12½ per cent of their revenues.

6. The Legislative enactment which contains most of the provisions regarding notification and control of epidemic diseases is the Madras Public Health Act of 1939.

(a) As regards notification of Infectious Diseases, all doctors must notify cases of infectious diseases to the Health Authority or in the case of rural areas to the Village Headman ; similar obligation rests on the householders when a direction to that effect is issued by the Collector of the District. Whilst a certain proportion of the cases is notified by the doctors and the public, the rest are detected by the health staff in the case of towns and the Village headmen in the case of rural areas ; as regards the latter areas, the health staffs also discover an appreciable number of missed cases during their visits to the villages. The Village Headman sends a copy of the epidemic report to the Tahsildar and another copy directly to the Health Inspector in order to enable the latter to take immediate preventive and control measures. The Tahsildar sends daily to the Director of Public Health, District Health Officer and other specified officers in the district a consolidated return for the infected villages in his jurisdiction. In the case of Municipalities, these daily returns are sent to the Director of Public Health by the Municipal Health Officers or Executive Authorities. The returns for the Province are compiled in the Director of Public Health's office in the Epidemic section under the immediate control of an Assistant Director. There are four other Assistant Directors for other subjects.

(b) The Madras Public Health Act confers powers on Government to appoint Special Health Officers in the areas affected by or threatened with epidemics. The Director of Public Health also possesses powers in connection with prevention and control of epidemics. In addition to giving general directions to local bodies on the above subject, he may assign to any local area the public health personnel from other areas, whether these belong to Provincial or Local Board services. Health Officers have inherent powers under the Public Health Act to appoint additional staff, concentrate in an area members of the staff from other places in their jurisdiction, purchase medicines and equipment, carry out disinfection measures etc., the last of these powers is conferred on Health Inspectors also. When the Collector of the District notifies an area to be affected by or threatened with an epidemic of any of the scheduled diseases of which cholera is one, the Health Officer gets additional powers, viz., control of movement of population and goods, disinfection, closure of markets, enforcement of inoculations and vaccinations and other kindred measures. It may also be

stated in this connection that rules to supplement these measures have been framed in several instances by Government and these rules have the force of law.

In the case of the epidemic in 1942-43, the powers conferred by the Public Health Act were fully availed of for the adoption of measures for prevention and control. All available resources in men and material were pooled and concentrated wherever they were required. Besides the additional staff, the services of private medical practitioners and institutional and subsidized rural medical personnel were fully utilized in control work. Incidentally, this demonstrates the value of close liaison between the Public Health and Medical Departments. Anti-cholera inoculation was declared compulsory. The holding of fairs and festivals was either completely banned or permitted under due safeguards according to the exigencies of the situation.

Epidemiology of cholera.

7. Next in importance to malaria, cholera is the most important of the endemic and epidemic diseases in the Province, especially from the standpoint of mortality. In the period of 69 years from 1874 to 1943, the Province has not experienced even a single year of freedom from cholera. The severity of infection varies from year to year ; this will be seen from the following summary.

Less than	5000		deaths	in a year were recorded in		5	years.
	5000	to	10000	do.	do.	5	do.
	10000	to	20000	do.	do.	8	do.
	20000	to	30000	do.	do.	10	do.
	30000	to	40000	do.	do.	12	do.
	40000	to	50000	do.	do.	4	do.
	50000	to	75000	do.	do.	10	do.
	75000	to	100000	do.	do.	7	do.
	100000	to	150000	do.	do.	7	do.

In 1877 when there were acute and widespread famine conditions throughout the Province, 357,437 deaths from cholera were recorded.

8. Russell's findings on the epidemiology of cholera are amplified below in the light of subsequent observations :

(i) Famine conditions which may be caused by failure of the monsoon or by excessive or unseasonal rains resulting in damage to standing crops, congregations at fairs and festivals, movement of labour populations at certain seasons for agricultural employment, especially in connection with transplantation and harvest, congregation of relatives and friends on occasions of marriages and funerals associated with deaths from cholera, may give rise to localised epidemics from which infection may spread to adjoining areas or be transferred to distant areas.

(ii) There is a close correlation between monsoons and cholera incidence :

In the northern group of districts and the south-west coastal districts, cholera epidemic synchronizes with the South-West monsoon.

In the southern districts which derive their rainfall from the North-East monsoon, the peak of the cholera wave is reached two months after the advent of the monsoon.

The central districts get their rainfall from both these monsoons. In this group, there are two peaks in the cholera curve. These are reached a few weeks after the commencement of the monsoons. The winter peak is more pronounced than the summer peak. This double peak is the resulting effect of two distinct epidemics in these districts. In the deltaic areas, i.e., the areas which are irrigated by the rivers, and their channel systems, the North-East monsoon or the winter epidemic prevails; in the non-deltaic areas, the South-West monsoon or the summer epidemic prevails. When once the cholera epidemic starts, the cycle of events is fairly regular. The winter epidemic begins by about the end of November or beginning of December, reaches its maximum virulence in January and then gradually subsides about the end of February. The infection does not however die out completely but continues in sporadic form during March, April and May in the villages bordering on the deltaic area. The summer epidemic of the dry area commences in June, reaches its maximum intensity in July or August and subsides about the end of September or beginning of October. The infection continues more or less in a sporadic form during October and November after which the next winter epidemic commences.

(iii) Apart from the seasonal incidence every year, there is a definite periodicity of cholera in Madras Province at intervals of about every six years.

(iv) In the case of the large widespread cholera epidemics in the Province, infection is invariably introduced from two sources, viz., from Orissa via Vizagapatam and from Hyderabad or/and Bombay through Bellary and Kurnool districts. Whilst in the former case the infection does not traverse far, in the latter case it spreads rapidly South-East and South.

(v) The following extract from the report of the District Health Officer, South Arcot, which is one of the districts included in the survey, will be found to be of interest :

" Generally in all the taluks, there were two distinct rises in the trend of the epidemic. The first phase was within three weeks from the date of onset, and another along with the usual winter epidemic, commencing from the middle of December, 1942 to the first half of January, 1943. The second phase of the epidemic is the result of the part played by the groundnut pickers (migrating agricultural labourers). The labouring classes who leave their houses in search of livelihood to taluks where groundnut produce is in harvest and return home by the end of December, carried with them the infection. Further, these labourers spend their time in groundnut fields, eat freely raw groundnuts and take water from any source available and sleep in open air and expose themselves to the influence of the weather. To quote one instance, cholera infection in Villupuram taluk was confined to villages adjoining the river Pennar and it was subsiding. But on the 16th November, 1942, there was a sudden outbreak of cholera in Viramur village in a gang of groundnut pickers. These labourers fled away in panic in different directions while suffering from or incubating the disease. Information was received by this department only when the coolies had run away. This gang was responsible for spreading the infection in 22 villages in Villupuram taluk and in addition caused infection to be spread in Gingee taluk which had been free from the epidemic in all these months."

More or less similar features apply to epidemics in several other districts.

8. The previous periodic wave of cholera was in 1935-36 when practically every district in the Province was affected and 70,000 deaths were recorded.

The present widespread periodic wave of the epidemic commenced late in the first quarter of 1942 in Bellary in the Ceded districts where there were acute water scarcity and famine conditions at the time. Outbreaks of cholera also occurred during the period in two festival centres in Bellary and Kurnool districts and the returning pilgrims contributed further to the spread of infection. This was still further fostered by monsoonal conditions and the infection ultimately travelled into the central and south-east coastal districts. With the exception of about half a dozen districts in the North-East coastal zone, the rest of the Province was affected by the ' periodic ' wave which continued into 1943. The recorded mortality from cholera in 1942 and 1943 was approximately 53,000 and 1,17,000 respectively. This mortality has been exceeded on six previous occasions. The epidemic in the present periodic wave would not have been so widespread and severe, were it not for the aggravating factors of famine conditions in certain districts, unseasonal rains and very heavy rains causing extensive flooding and gross contamination of water supplies and damages to food crops, large movements of population, general food scarcity, economic distress, difficulties in the transport of staff and equipment, shortage of medicines and disinfectants and other similar handicaps.

Present enquiry.

9. In the course of the preliminary discussion regarding the lines of enquiry, a suggestion was made that the best data would be those secured on an experimental basis by carrying out anticipatory inoculations in certain villages and by leaving certain other villages under more or less identical conditions wholly unprotected. This was not however possible for several reasons. In the first place, it is only on the actual occurrence of cholera in a village or in the immediate neighbourhood that the public volunteer or can be persuaded to be inoculated. Secondly, when there is a demand for inoculation, it would be impracticable for administrative reasons to inoculate one section of the population, and leave the other section uninoculated simply for the sake of an experiment. Thirdly, on account of the widespread nature of the epidemic, the available field staff and vaccine supplies were just sufficient to meet the needs of the areas actually affected. It may however be stated in this connection that, in the present epidemic, several villages experienced more than one outbreak of cholera. The inoculations done in the first epidemic in these villages amount virtually to anticipatory inoculations with respect to the subsequent outbreaks in the same village.

10. According to the rules now in force, the headman of every village should give prompt information to the health staff of the occurrence of every cholera case in the village. He should also maintain a register wherein particulars of the cases with respect to name, age, sex, date of attack, and date of death in fatal cases should be recorded. The Health Inspector is required to maintain among other records, a register of the name, sex, age and address of the persons inoculated, and date of inoculation. As the village cholera registers do not contain information whether the cases had been previously inoculated, Collectors of Districts were requested to direct village headmen to enquire and record in the register in respect of every cholera case whether the person was previously inoculated or not, and if inoculated, the date of inoculation. As village headmen are intimately acquainted with the people of villages, the particulars recorded in the cholera registers are generally accurate. These entries were

further checked by the Health Inspectors by a reference to the inoculation registers.

11. The data collected in the survey were entered on cards specially designed and printed for the purpose. These cards contain all the particulars considered necessary for the enquiry and could be easily filled in, from the registers referred to in the previous paragraph. They were distributed to the Health Officers of eleven districts, viz., South Arcot, Madura, Tanjore, Trichinopoly, Tinnevelly, North Arcot, Chingleput, Bellary, Anantapur, Cuddapah and Kurnool. According to the distribution of infection in the village, the whole village or affected hamlet was taken as a unit and the particulars of each unit, were recorded on a separate card. Every card received from the health staff was scrutinized and discrepancies and omission rectified before it was included in the compilation.

The Municipal towns in the eleven districts were excluded from the survey, as the conditions therein are different from those in the rural areas.

The total number of cholera-infected villages and hamlets for which data was collected and compiled was 2,350. The total incidence of cases and deaths in these was approximately 55,500 and 30,200 respectively. The population exposed to risk was about 2.99 millions and of these 1.18 millions were inoculated.

12. The serological type of *V. cholerae* prevalent in different outbreaks in the several districts of the Province has been continuously studied since 1936 by Dr. K. V. Venkatraman at the King Institute, Guindy. As a result of the examination of nearly 4,000 strains of *V. cholerae* during the period, it was found that, while in the earlier years, there was a clear demarcation of the areas of prevalence of Inaba and Ogawa types, the majority of strains in the outbreaks following 1940 throughout the Province have been Ogawa. Both types of *V. cholerae* are included in the prophylactic vaccine made at the Institute which has been used in the control of the epidemic under investigation.

13. The greater proportion of the inoculations was done by Sanitary Inspectors trained in inoculation work ; only a small proportion of inoculations was performed by qualified medical men.

14. In the great generality of instances, the vaccine was administered in a single dose as this alone is possible in mass inoculation campaigns during epidemics. In the case of adults, the dosage was 1 c.c. containing 8000 million vibrios ; half this dose was given in the case of children.

15. The following is a brief summary of the scope of the enquiry :

(a) Whether anti-cholera inoculation confers immunity against cholera, and if it does, the time taken for its development from the date of inoculation. At present, on the assumption that inoculation confers immunity, an international standard of six days has been prescribed as the period for the development of the immunity. The data required for determining the above are :

(i) the relative incidence of cholera in the inoculated and the uninoculated groups of the population exposed to risk and

(ii) the incidence of cholera in the inoculated group of population exposed to risk classified according to the occurrence of the cases on the days succeeding the date of inoculation.

(b) The duration of the immunity, if any, conferred by inoculation ; this could be determined from the incidence of cholera among the inoculated persons in the populations at risk in villages which experienced more than one outbreak of the disease when the interval between such outbreaks is not unduly short.

(c) The influence if any of mass immunity on the duration of the epidemic and on the occurrence of subsequent outbreaks within a reasonable interval. This could be determined from the data in (a) and (b) above.

(d) Relative sex and age incidence of cholera.

16. For the purpose of the present study, the following definitions have been adopted :

(i) No prophylactic vaccine gives instantaneous immunity. Varying periods are required for the development of a sufficiently high level of immunity. The period of six days prescribed in the International Quarantine Regulations for considering as " protected " a person inoculated with anti-cholera vaccine has been adopted for the differentiation of persons " protected " by inoculation from the rest of the population at risk.

(ii) In the case of villages which experienced more than one outbreak of cholera, the epidemics were considered as separate and distinct if the interval between the successive outbreaks was 30 days and above. This is in order to avoid any fallacies that might arise on account of missed or sub-clinical cases.

Alternative classifications with two weeks, three weeks and 30 days intervals were also tried in 163 villages in Villupuram range in South Arcot district ; the results of such classification do not show any material difference from the method that has been finally adopted.

(iii) The duration of an epidemic in a place is defined as the number of days, both inclusive, elapsing from the date of the first to the date of report of the last case in the outbreak.

(iv) If the distribution of cases of cholera was more or less general in a village, the census population of the village was considered as the population at risk. If the incidence of cases was restricted to one or more hamlets only, the population of these hamlets as determined by rough enumeration was taken as the population at risk.

STATISTICAL ANALYSIS OF THE DATA COMPILED DURING THE SURVEY.

17. The conclusions arrived at from the analysis of the statistical data in the four-fold tables in the succeeding paragraphs are based on KARL PEARSON'S X^2 values and the corresponding 'P' values in the X^2 tables, the degree of freedom being 1. The 5 per cent level was adopted in testing for the significance of the observed deviations.

In the case of percentages, the difference in proportion was tested by comparing it with the standard error given by the formula—

$$\text{Standard error} = \sqrt{pq\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

where p, q, n_1 and n_2 have the usual interpretation. If the difference in proportion was greater than twice its standard error, it was taken to be significant.

18. WHETHER INOCULATION CONFERS IMMUNITY FROM CHOLERA.

This can be determined in one of two ways. In the first method, the incidence of cholera among the uninoculated in the whole of the experience after commencement of inoculation may be compared with the corresponding rate in the 'protected' population as defined in paragraph 16(i) supra. As an alternative and more refined method, the incidence of cholera among the uninoculated persons during the second outbreak in the villages which experienced more than one outbreak of cholera in the epidemic may be compared with the incidence among the persons inoculated upto six days prior to the second outbreak. These come within the category of anticipatory inoculations.

(2) As regards the first of the above methods, it has to be pointed out that, in the great majority of villages, the inoculations were not done on a single day but were spread out over a number of days. The proportion of the 'protected' to the 'uninoculated' persons varied therefore from day to day and the period of exposure of these two groups to risk of infection in the course of the epidemic was changing. In order to make allowance for this, the weighted means of the protected and uninoculated persons were taken as the populations at risk in each group, the weights used being the days of exposure to infection. The weighted mean 'protected' population was 709, 977 and the weighted mean 'un-inoculated' population was 2,119,568. The inoculated persons who had not completed the six day period after inoculation have been excluded in order to eliminate the fallacies that might arise as a result of partial immunity.

The number of cases of cholera among the 2,119,568 uninoculated persons and 709,977 protected persons was 34,336 and 1118 respectively. These correspond to 16.20 and 1.57 per 1000 population in each group. The incidence rate of cholera in the uninoculated is 10.3 times the rate among the 'protected.' These figures are subjected to further analysis in the following table :

Table I.

	Not attacked.	Attacked.	Total.
'Protected' population	708,859	1,118	709,977
Uninoculated population	2,085,232	34,336	2,119,568
Total	2,794,091	35,454	2,829,545

The value of X^2 is 9135.09 and that of P is < 0.0016. The difference in incidence rates in the two population groups is significant.

(3) Out of a total number of 2,350 infected villages, 627 villages experienced more than one outbreak of cholera in the epidemic. In the latter group of villages, 541,808 persons were uninoculated and 281,484 were 'protected'; excepting a negligible fraction, the rest of the 'protected persons' had been inoculated in the first outbreak; these inoculations may be considered as 'anticipatory inoculations' with reference to the second and subsequent outbreaks. The inoculations done in the second outbreak are excluded from consideration. The incidence of cholera was 6,580 in the 541,808 uninoculated persons and 241 in the 281,484 'protected' anticipatory inoculations representing rates of 12.14 and 0.86 per 1000 in the respective populations. The incidence

rates in the uninoculated persons was 14.2 times the rate in the anticipatory inoculations ; the corresponding figure arrived at in the previous method is 10.3.

The figures of incidence of cholera in the two groups of population are subjected to further analysis :—

Table II.

	Not attacked.	Attacked.	Total.
Number protected	281,243	241	281,484
Number unprotected	535,228	6,580	541,808
Total	816,471	6,821	823,292

$X^2 = 2875.05$ and $P = 0.0016$

The difference is therefore significant.

(4) These inferences are not in any way vitiated by an undue weightage of sex and age composition of population in either of the two groups. There was no selection of any kind among the people who were inoculated as inoculations were done irrespective of age and sex among the people who came up for inoculation ; also, among the general population, there were no objections to inoculation on the score of age and sex. That there was no bias in selection may be recognised from the age and sex incidence of cholera and age and sex distribution of the population in 100 randomly chosen villages (according to Tippets Random Numbers) for which data are available.

Age Groups	No. of attacks.			Percentage to Total.			% of population to total.		
	Male.	Female.	Total.	Male.	Female.	Total.	Male.	Female.	Total.
Under 5 years	121	106	227	11.5	9.6	10.6	14.2	14.6	14.4
5–14	237	276	513	22.6	25.1	23.9	24.8	23.7	24.2
15 and above.	691	718	1409	65.9	65.3	65.5	61.0	61.7	61.4
Total	1049	1100	2149	100	100	100	100	100	100

It will be seen from the above that the age and sex distribution of cholera does not materially differ from the age and sex proportions of the population.

(5) Whilst the incidence of cholera among the 'uninoculated' is about 10 to 14 times the rate in the ' protected ' population, it is worth while considering if the immunity conferred by the inoculation reduces the risk of death from cholera when a ' protected ' person gets cholera. Taking the whole experience into consideration, there were 50,053 cases of cholera and 27,576 deaths among the uninoculated population, the case fatality rate being 55.1%. Among 1,118 cases of cholera in the ' protected', there were 474 deaths (42.4%). The case fatality in the ' uninoculated' persons is 1.3 times higher than in the ' protected ' group of population.

Table III.

	Survivals.	Deaths.	Total.
Attacks among the ' protected '	644	474	1,118
Attacks among the uninoculated	22,477	27,576	50,053
Total	23,121	28,050	51,171

Applying the X^2 test, $X^2 = 71.11$ and P is < 0.0016. The difference is therefore significant. From the foregoing discussions, it is established that, whilst the 'protected' are twelve to fourteen times less susceptible to an attack of cholera than the 'uninoculated', their chances of recovery when they get the disease are only 25 per cent more than the uninoculated.

19. TIME REQUIRED FOR THE DEVELOPMENT OF IMMUNITY.

(i) Having established that anti-cholera inoculation affords immunity against cholera, the next question for consideration is the time required for the development of this immunity. This can be determined by comparing the daily cholera incidence rates amongst the inoculated persons commencing from the date of inoculation. From the beginning to the end of the epidemic in the 2350 villages under survey, the total number of persons inoculated was 1,181,724. The incidence of cholera in this group according to the day of its occurrence after inoculation is given in the following table :—

Table IV.

S. No.	Interval in days after inoculation.	Number inoculated at risk.	Number of attacks in inoculated.	Incidence rate per 10,000	X^2	P.	Remarks.
1	2	3	4	5	6	7	8
1.	Same day	1,181,724	370	3·13			
2.	1st day	1,181,354	844	7·14	1·47	0·23	Not significant.
3.	2nd day	1,180,510	894	7·57	5·62	0·02	Significant
4.	3rd day	1,179,616	796	6·75	21·01	<0·0016	do.
5.	4th day	1,178,820	623	5·28	20·19	do.	do.
6.	5th day	1,178,197	474	4·02	37·07	do.	do.
7.	6th day	1,177,723	304	2·58	19·69	do.	do.
8.	7th day	1,177,419	204	1·73	18·97	do.	do.
9.	8th day	1,177,215	125	1·06	19·69	do.	do.
10.	9th day	1,177,090	64	0·54	0·03	0·86	Not significant.
11.	10th day	1,177,026	66	0·56			
12.	11th day and more.	1,176,960	659	5·60			

The inoculated population at risk on each day (column 3 of the table) is calculated by deducting the number of cases which occurred the previous day from the population. Before discussing the figures contained in the table,

it has to be pointed out that 370 cases which occurred on the day of the inoculation should be left out of consideration. The inoculations were done from morning to night on each day and the time interval between inoculation and the development of cholera on the day of inoculation is so short that any inferences that would be drawn will be fallacious. In the greater proportion of these cases, the inoculated person was already on the way of manifesting symptoms of cholera and inoculation could not have influenced it. This remark applies to some extent to a certain proportion of cases occurring on the day following the inoculation. It is not uncommon for the staff to do the inoculations even until midnight, and the interval between the hour of inoculation and the manifestation of cholera symptoms in the cases which occurred on the following morning among the inoculated group is so short that the occurrence of the disease in such individuals could not have been influenced by the inoculation. Next, as regards the 659 cases which have been recorded as having occurred on the 11th and subsequent days after inoculation, it has to be stated that these also should not be compared with the case incidence on the previous days; whilst the latter represents the incidence on individual days, the 659 cases relate to the total experience of 174 days during which period these cases occurred and comparison would therefore be fallacious.

(ii) It will be seen from the incidence rates in column 5 of Table (iv) that, after the exclusion of the figures relating to the day of inoculation, there is no appreciable difference in the rates recorded on the first and second day following the inoculations. On the third day, there is an appreciable decline which continues into the 4th and 5th days and becomes more pronounced on the succeeding days. The values of X^2 and P in columns 6 and 7 of the table (iv) demonstrate the significance of the differences in incidence rates.

Although a day to day analysis of the 659 cases of cholera which occurred on the 11th and subsequent days has not been attempted, the following classification has been made of the incidence of cases in the population under consideration each week in order to find out the trend of incidence :

Table V.

Period.	Number inoculated at risk.	Number of attacks.	X^2	P.	Remarks.
Same day.	1,181,724	370			
1 to 3 days.	1,181,354	2534			
4 to 10 days	1,178,820	1860	689.66	<0.0016	Significant.
11 to 17 „	1,176,960	172	31.89	<0.0016	do.
18 to 24 „	1,176 788	82	14.46	„	do.
25 to 31 „	1,176,706	40	0.73	0.39	Not significant.
32 to 38 „	1,176,666	48	5.26	0.02	Significant.
39 to 45 „	1,176,618	28	0.07	0.79	Not Significant.
46 to 52 „	1,176,590	27	1.03	0.31	do.
53 to 59 „	1,176,563	35	(Diffce. in rates is negligible)		
60 to 66 „	1,176,528	35	1.33	0.25	Not significant.
67 to 73 „	1,176,493	26	0.16	0.69	do.
74 to 80 „	1,176,467	29	0.07	0.79	do.
81 to 87 „	1,176,438	27	3.43	0.06	do.
88 to 94 „	1,176,411	15	1.32	0.25	do.
95 to 101 „	1,176,396	22	5.45	0.02	Significant.
102 to 108 „	1,176,374	9		(Diffce. negligible)	
109 to 115 „	1,176,365	9	1.50	0.22	Not significant.
116 to 122 „	1,176,356	15	0.33	0.57	do.
123 to 129 „	1,176,341	12			
130th to 185th day	1,176,329	28			

It would seem from these figures that the quantum of immunity developed between the 4th and 10th day after inoculation increased still further during the subsequent five weeks ; and the significance of the difference is confirmed by the results of statistical analysis. The figures for the subsequent weeks, are not statistically significant.

(iii) In this connection, it would be worth while comparing the daily cholera incidence rates among the inoculated persons in the first few days of inoculation with the rates among the uninoculated persons on the corresponding days. This has been done in the following table with reference to the first batch of 412, 438 inoculations.

Table VI.

Period.	Inoculated.			Uninoculated.			X^2	P	Signi-ficance.
	Populn. at risk.	Atta-cks.	Rate per 10,000	Populn. at risk.	Atta-cks.	Rate per 10,000			
1	2	3	4	5	6	7	8	9	10
Same day	412,438	183	4.44	1,640,436	2224	13.56	—	—	—
1st day	412,255.	438	10.62	1,638,212	1842	11.24	1.14	0.29	Not Significant.
2nd day	411 817	471	11.44	1,636,370	1710	10.45	3.02	0.08	do.
3rd day	411,346	435	10.58	1,634,660	1738	10.63	0.01	0 92	do.
4th day	410,911	328	7.98	1,632,922	1614	9.88	12.49	0.0016	Significant.
5th day	410,583	250	6.09	1,631,308	1445	8.86	30.30	< 0.0016	do.
6th day	410,333	156	3.80	1,629,863	1342	8.23	87.78	< 0.0016	do.
7th day	410,177	127	3.10	1,628,521	1354	8.31	122.93	< 0.0016	do.

Excluding the figures relating to the day of inoculation from consideration for reasons stated in sub-para (i) above, it will be seen from the table that, as compared with the uninoculated, whilst there is no appreciable difference in the incidence rates in the inoculated persons in the first three days of inoculation ; the rates are definitely less on the 4th to 7th day, the actual differences being 1.90 on the 4th day, 2.77 on the 5th day, 4.43 on the 6th day and 5.21 on the 7th day, when according to Table (VI), a high level of immunity is reached. This is substantiated by X2 test, the X2 and 'p' values being given in columns 8 and 9 in Table (VI). It will also be seen from the figures in columns 4 and 7 of the table that there is a progressive daily decline in case incidence from the 2nd to 7th day in the inoculated as well as the uninoculated persons due probably to a decline in the severity of the epidemic or to the influence of a population aquiring an increasing herd immunity. The decreases in the trends in the two groups of the population are not however parallel. Whilst the decline in the daily cholera incidence rate from the 3rd to the 7th day is 70.80 per cent in the inoculated persons, it is only 22.09 in the uninoculated group. This striking difference cannot but be attributed to the effect of immunity conferred by inoculation.

(iv) The requirements of the International Sanitary Convention presuppose that effective immunity after cholera inoculation is reached in six days. An analysis of the data in the present survey shows that even on the 3rd day

after inoculation, a fairly high level of degree of immunity is attained and this continues to increase during the subsequent days and in the next few weeks. This is of significance from an administrative point of view. Anticipatory inoculations would be the best precautionary measure for the individuals as well as the community; for reasons already stated, this measure is however not generally feasible of adoption in communities. The next best thing would therefore be to carry out an intensive inoculation campaign on the occurrence of the outbreak. For obvious reasons, the earlier this campaign is commenced, the more effective it will be in the control of the epidemic.

20. DURATION OF IMMUNITY CONFERRED BY ANTI-CHOLERA INOCULATION.

(i) This may be determined by one of two methods. The first of these is by studying the relative incidence of cholera in the uninoculated and the inoculated groups in a community in the course of an epidemic lasting for a sufficiently long time and noting the interval between the occurrence of cholera in the inoculated and the dates of inoculation. It has however to be pointed out that an epidemic does not generally continue for such long periods in a locality as to enable such studies being made. This may be recognized from the facts that, in the villages under survey, the mean duration of the outbreak was only 19 days which is unduly short for the study. The second method is the study of the relative incidence of cholera in communities comprising sufficiently large numbers of inoculated and uninoculated persons, the inoculations having been done at varying periods before the occurrence of cholera in the communities. It has been stated already that in the present epidemic, there have been more than one outbreak of cholera in certain villages; the interval between the first and the second outbreak in these villages has ranged from one to six months or more. The occurrence of cholera cases among the inoculated and the uninoculated groups of population in the second outbreak gives exceptionally good data for determining the duration of immunity conferred by inoculation. Before considering these data, reference may be made to an alternative set of figures, viz., the cases of cholera among the inoculated group of population at varying intervals after inoculation.

The following statement shows the incidence of cholera in four-weekly periods commencing from the fourth day after inoculation; the latter day has been chosen as the effects of immunity are noticed on that day.

Period.	Number of cases of cholera in the inoculated group.
4th to 31st day after inoculation.	2154
32nd to 59th do.	138
60th to 87th do.	117
88th to 115th do.	55
116th to 143rd do.	42
144th to 171st do.	11
172nd to 199th. do.	2

The large fall in the cases from the first four-weekly period to the second is striking; the progressive tailing off of the cases in the subsequent weeks and the absence of cases after the 199th day is a rough indication of the duration of

immunity up to a minimum of about seven months. These figures would have had additional significance if the data of cholera cases in the uninoculated population in the corresponding period had been shown side by side. But in view of the data in Table VI in the previous paragraph, it is extremely improbable that there would have been no cases during the period in this group or that the proportional incidence of cholera in this group would have been less than in the inoculated group.

(ii) Table VII gives details of the cases of cholera in the populations of villages which experienced a second outbreak. The inoculated group represents the persons inoculated in the first outbreak. The villages are classified according to the intervals which elapsed between the first and second outbreak. It will be seen from the table that upto an interval of five months between the first and second outbreaks, the incidence rates in the inoculated are definitely less than the corresponding rates in the uninoculated and statistical analysis shows that the differences in rates are significant. As regards the succeeding months, whilst the incidence rates in the uninoculated ranged from a minimum of 5.13 to a maximum of 14.19 per 1000 population of the group, the number of cases of cholera in the inoculated persons was one in each of the intervals 5 to 6 and 6 to 7 months and nil in the five succeeding months ; although a statistical analysis would be fallacious on account of the smallness of these figures, the latter by comparison with the case incidence rates in the uninoculated group during the period, strongly point to the presence of immunity in the inoculated population definitely upto ten months and probably also upto twelve months after inoculation.

Table VII.

S. No.	Interval between two outbreaks.	No. of persons inoculated in first outbreak.	Attacks among inoculated in 2nd outbreak.	Rate per 10,000 inoculated.	Number inoculated.	Attacks among uninoculated.	Rate per 10,000 uninoculated.	X^2	P.	Remarks.
1.	1 month-2 months	139,006	129	9.3	247,519	3,317	134.0	1566.8	< 0.0016	*
2.	2-3 months	64,845	72	11.1	133,998	1,509	112.6	571.1	< 0.0016	*
3.	3-4 do.	42,238	31	7.3	83,368	1,237	148.4	558.0	< 0.0016	*
4.	4-5 do,	17,635	7	4.0	37,181	285	76.7	119.2	< 0.0016	*
5.	5-6 do.	8,909	1	1.1	16,932	76	44.9			
6.	6-7 do.	650	1	15.4	4,070	32	78.6			
7.	7-8 do.	1,769	..	..	7,352	44	59.8			
8.	8-9 do.	279	..	..	1,264	8	63.2			
9.	9-10 do.	5,748	..	..	7,454	50	67.1			
10.	10-11 do.	227	..	..	1,754	9	51.3			
11.	11-12 do.	178	..	..	916	13	141.9			

N.B.-(*Star mark in the last column of the statement denotes " SIGNIFICANT ".

21. INFLUENCE OF HERD IMMUNITY ON THE OCCURRENCE OF SUBSEQUENT OUTBREAK OF CHOLERA.

(i) With reference to the proportion of populations inoculated, classification of the populations of the villages which experienced one outbreak and those which experienced more than one outbreak of cholera gives the following results :

Table VIII.

	Total Population.	No. of persons inoculated prior to II outbreak.	Proportion of population inoculated.
Villages with one outbreak	2,038,608	808,849	39.68 %
Villages with more than one outbreak	948,154	281,484	29.68 %

The difference in the two proportions is $0.3968 - 0.2968$ or 0.1000

Standard error of the difference in the proportions

$$= 0.365 \times 0.635 \left(\frac{1}{2,038,608} + \frac{1}{948,154} \right) = .0006$$

$$\therefore \frac{\text{Difference}}{\text{Standard error}} = \frac{0.1000}{0.0006} = 167.$$

The difference is therefore significant.

It is seen from these figures that the proportion of inoculations in a community influences the occurrence of subsequent outbreaks:

(ii) The extent to which the proportion of inoculations done in the population influences the occurrence of second outbreak is shown in greater detail in the following table:

Table IX.

Percentage of population inoculated before second outbreak:	No. of villages with one outbreak.	No. of villages with two outbreaks.	Total No. of villages.	Percentage of villages with two outbreaks to total.
0 and less than 10	93	86	179	48.0
10 „ 20	173	99	272	36.4
20 „ 30	196	124	320	38.8
30 „ 40	226	100	326	30.7
40 „ 50	216	83	299	27.8
50 „ 60	240	57	297	19.2
60 „ 70	204	37	241	15.4
70 „ 80	163	20	183	10.9
80 „ 90	99	11	110	10.0
90 „ 100	113	10	123	8.0
Total ..	1723	627	2350	..

The influence of the percentage of populations inoculated on the occurrence of second outbreak may be tested by applying the method of the analysis of variance to the above table ;

ANALYSIS OF VARIANCE.

Table X.

	Sums of squares.	Degree of freedom.	Variance.	Z.
Between villages	986.18	1	986.18	2.57
Within villages	13,669.19	2348	5.82	
Total	14,655.37	2349	..	..

The value of Z is significant.

In a widespread epidemic of prolonged duration as the one under consideration, herd immunity plays an important part in preventing subsequent outbreaks and this has administrative significance.

22. INCIDENCE AND SEVERITY OF CHOLERA IN RELATION TO WATER SUPPLIES.

The sources of drinking water-supplies in the villages in the survey comprised one or more of the following:—viz. wells, ponds, tanks, rivers and streams. Even as regards the first of these, the great majority of them could not, on account of the nature of their construction and maintenance, be considered as 'protected' sources. This remark applies with far greater force to the remaining sources of supply which could neither be satisfactorily conserved nor disinfected. In these circumstances, the analyses of the cases of cholera in relation to type of drinking water supplies did not lead to any definite conclusions.

SUMMARY AND CONCLUSION.

23. (i) A widespread and severe cholera epidemic involving the major portion of the Province of Madras prevailed in 1942 and 1943. The mortality from cholera in the two years was 53,000 and 117,000 respectively.

(ii) One of the chief measures of prevention and control adopted by the Public Health Department of the Province was anti-cholera inoculation. Opportunity was taken of the very large number of inoculations done during the epidemic to carry out an investigation of certain aspects of inoculations in the prevention and control of cholera. The investigation was carried out in 2,350 villages in eleven districts of the Province. The total population of these villages was 2.99 millions of whom 1.18 millions were inoculated with prophylactic vaccine containing both Inaba and Ogawa types of *Vibrio cholerae*. The vaccine was manufactured in the King Institute of Preventive Medicine and was administered in a single dose of 1 c.c.

(iii) According to the standard in the International Quarantine Regulations, a person to whom anti-cholera vaccine has been administered is deemed to be protected after six days have elapsed from the date of inoculation. Excluding the inoculated persons who did not satisfy the standard, there were 709,977 'protected' persons in whom 1,118 cases of cholera occurred; in 2,119,568 uninoculated persons, there were 34,336 cases of cholera. The case incidence rates in these two groups of population were 1.57 and 16.20 respectively per

1,000. The incidence rate in the uninoculated was 10.3 times more than in the 'protected' group.

627 out of 2350 villages in the survey experienced a second outbreak of cholera after a month or more subsequent to the last case in the first outbreak. In these re-infected villages, the uninoculated population in the second outbreak was 541,808 whilst the protected population was 281,484, the greater proportion of whom had been inoculated in the first outbreak. In the second outbreak, 6,580 cases of cholera occurred in the uninoculated group and 241 in the 'protected' group. The incidence rates per 1,000 of the respective population groups were 12.14 and 0.86. The incidence rate in the uninoculated was 14.2 times greater than in the 'protected' group.

From the two foregoing sets of results, there is definite proof that the protected persons are 12 to 14 times less susceptible to getting cholera, than the uninoculated persons in the community.

(iv) In the whole experience, there were 1,118 cases and 474 deaths from cholera in the 'protected' group and 50,053 cases and 27,576 deaths in the uninoculated group. The case fatality rates in the two groups are 42.4% and 55.1% respectively, the proportion being 10 : 13.

The advantage derived from anti-cholera inoculation is not so much in recovery from the disease as in not getting it.

(v) According to the International Sanitary Convention, anti-cholera inoculation affords effective immunity only six days after the date of inoculation. As judged from the daily incidence of cholera in 1,181,724 inoculated persons on the several days following inoculation, it is seen **that immunity is established even on the third day after the date of inoculation and it increases progressively during the succeeding week as well as the next four weeks.**

(vi) 627 out of 2,350 villages experienced a second outbreak of cholera, the intervals between the last case in the first outbreak and the first case in the second outbreak ranging from 1 to 12 months. If these re-infected villages are classified according to the intervals that have elapsed between the first and second outbreaks and the cholera incidence in the second outbreak among the uninoculated persons and the persons who had been inoculated in the first outbreak, is studied, the duration of immunity can be determined. From such a study, there is definite **proof that the immunity conferred by anti-cholera inoculation lasts for a minimum period of FIVE months and there is also strong evidence pointing to the continuance of the immunity up to 10 to 12 months.**

(vii) From the data relating to the villages which experienced more than one outbreak of cholera in the present epidemic, there is definite evidence that, the greater the proportion of inoculations done in the first outbreak, the less are the chances of an occurrence of a second outbreak. **Herd immunity therefore plays an important part during an epidemic on the occurrence of two or more outbreaks of cholera in a locality.**

MALARIA.

3. Malaria Institute of India, Research Section, Delhi and Coonoor.

The research activities of the Institute have again been severely curtailed owing to the abnormal conditions prevailing due to the war. The post of the Malaria Research Officer has remained unfilled, and a large proportion of the work of the staff has been devoted to instructional courses in malariology for officers deputed from the various branches of the Defence Department, by Provincial Governments, Railways and States. Five of these courses have been held during the year at which 127 officers have been trained. Special training in Entomology was arranged for one R.A.M.C., and two Naval Officers. Four malaria Supervisors of the Ordnance Factories were given practical training in malaria control.

Publications.

Journal of the Malaria Institute of India. Two numbers containing 10 papers and 5 abstracts have been published.

Scientific material sent to other workers.

Strains of monkey malaria have been supplied to workers in the U.S.A., and in Australia. A MISH type sprayer was also sent to Australia. Samples of J. S. B. stain were sent to the School of Tropical Medicine, Sydney, Australia and to a number of workers in India. Gambusia fish were supplied to a number of stations in India.

Testing of insecticides.

Indian Emulsifier (without pyrethrins) when diluted 1/20 possessed only very slight insecticidal properties. Water emulsion spray containing 0.025% pyrethrins prepared with the Indian emulsifier gave a kill of about 75% as compared to 100% obtained with a kerosene oil spray containing 0.1% pyrethrins. To obtain a 100% kill with the emulsion it is necessary to increase the pyrethrin content to 0.05%.

Sesame oil appeared to act as an activator for pyrethrins only when the temperature was above 85°F. and relative humidity below 50. Its effect was very much reduced when the humidity rose above 50. Pyrethrins appeared to be activated also by mustard oil and mobil oil. The latter gave considerably better results than mustard or sesame oil.

One Individual Sparklet Sprayer per 1500 c.ft. gave a 100% kill in the testing chamber and about 96% in the field when mosquitoes were exposed to the vapour for 20 minutes. It was observed that the time of discharge varied in different sparklets from 5 to 8 seconds.

Extracts of pyrethrum flowers grown at Quetta proved almost as effective as that prepared from Kenya flowers.

DDT water emulsion was tested in the field for its residual effect. A 5% emulsion sprayed on walls at 4 c.c. per sq. foot (or 200 mgm. of DDT per sq. foot) reduced the daily routine collection of mosquitoes from the room by 93 to 100%. This effect lasted about 9 days, after which the number of mosquitoes collected began to increase.

TESTING OF REPELLENTS AND PROTECTIVE CLOTHING.

Malaria Institute of India cream containing 0.2% pyrethrins repelled *culicifacies* for only 1 hr. but protected from bites for at least 4 hours. It protected against *fluviatilis* and *stephensi* for 2–3 hrs. Against *Aedes* it repelled for ½ to 1 hour and protected for 1—1½ hrs. A similar vanishing cream supplied by Messrs. F. S. Kerr & Co., said to contain 0.05% pyrethrins, repelled *Aedes* for 5 minutes only, but protected for 58 minutes.

DMP (dimethyl phthalate) when applied liberally repelled *culicifacies* and *stephensi* for about 3½ hours and protected for 4—5 hrs., but when applied in moderate quantity as is usually the case in practice the effect lasted only for 30 minutes. Against *A. fluviatilis* it afforded protection for 3½ to over 5 hours, and repelled *Aedes* mosquitoes for 1½ to 4 hours, but protected from biting for 1½ to 5 hours. DMP cream (containing about 60% dimethyl phthalate) repelled *Aedes* for ½ to 2 hours, and protected for 2 to 4 hours. Against Anopheles it protected from 2½ to 5 hours.

Rutgar 612 gave practically similar results as DMP. Deskito Mark VII repelled *Aedes* for only ¼ to 2 hours and gave protection from biting for ¼ to 4 hours. Against anopheles it protected for 3 to 5 hours.

When mosquito density was as low as 0.4 per c.ft. *A. culicifacies* was deterred from passing through 1½" mesh camouflage net irrespective of whether it was sprayed with DMP or not. Both fine and coarse mesh camouflage net when sprayed with about 12 to 14 mgm. of DMP per sq. inch acted as a deterrent to *culicifacies* when the density of mosquitoes was 20 per c.ft. but this protection failed when the density was increased to 50 per c.ft. It failed against *annularis*, however, at a much lower density. A fine fish net (mesh 1 cm. square) sprayed with DMP to the extent of half its weight, did not act as a deterrent to *Aedes* mosquitoes at least when it was away from the body. These mosquitoes readily passed through the sprayed net even when the net was kept at body temperature. *A. culicifacies* fed through cellular cloth even when silk underwear was worn beneath it. They did not feed through a shirt when sprayed with DMP in a strength of 4 mgm. per sq. inch. DMP sprayed on clothing was much more effective against *A. culicifacies* than against *A. annularis*.

The protection ratio afforded by American drill, Indian drill and cellular cloth was about 95, 80 and 50% respectively. A series of experiments with three different shirting materials received from the D.M.S. in India showed that although in each there was a considerable degree of protection a certain proportion of anophelines were able to bite through all these materials. Another series of experiments carried out with utility gaberdine made in U. K., Bombay cloth (two samples) and cellular cloth, showed that *Aedes* and *A. subpictus* are unable to bite through the utility gaberdine. In case of the other three samples there is a considerable degree of protection but a certain proportion of both *Aedes* and *A. subpictus* are able to bite through them.

TESTING OF LARVICIDES.

DDT (Dichlor-Diphenyl Trichlorethane) in used engine oil was tested in the field as a larvicide against anopheline and culicine larvae. 6 c.c. of 5% solution per 10 sq. yds. (=6 pints of solution, or 4½ oz. DDT per acre) appears to be the minimum lethal dose, producing a 100% kill of anopheline and culicine larvae when marginal vegetation was present. In the absence of marginal vegetation

4 c.c. per 10 sq. yds. was sufficient. The residual effect of such a dose lasted for 4–5 days when vegetation was absent but otherwise only for about 2 days. An oil film of DDT did not appear to inhibit oviposition after the 4th day.

Entomological research.

The study of mosquito bionomics in respect of the colonies maintained in the laboratories has been continued. Colonies of *Aedes aegypti* and *Aedes vittatus* have been maintained for experimental purposes. Laboratory bred *culicifacies* 12-40 hours old did not readily feed when the density of mosquitoes was only 0.4 per c.ft. The biting activity of *A. culicifacies* and *A. stephensi* gradually decreased after 01.00 hrs., the optimum being between 22-30 and 01.00. A number of collections of mosquitoes and other insects were received for identification from various parts of India.

Field researches.

Epidemiological studies have been continued in certain localities in the riverain section of Delhi urban area. Antimalaria operations in Delhi have been directed and supervised as in previous years.

Laboratory researches.

A number of monkey malaria strains have been maintained. High titre anti-human and anti-cow precipitin sera have been prepared from rabbits. Precipitin tests to determine the source of blood meals of mosquitoes were carried out on a number of specimens received from various workers in India and China.

A number of indigenous drugs received from India and Ceylon have been tested on rhesus monkeys infected with *Plasmodium knowlesi*, but none has been found to possess any antimalarial properties.

A number of blood smears from fever cases have also been examined.

Southern India branch at Coonoor.

Day-time resting places of A. fluviatilis. Intensive search in the most favourable natural and artificial shelters failed to reveal any adults of this species, except in human dwellings, although other mosquitoes both anopheline and culicine were present in such situations. Further observations were carried out on the two egg patterns of *A. fluviatilis*. It has been established that these are not hereditary.

The mode of digestion of mosquito blood meals has been studied. Observations on the influence of temperature on the number of blood meals required for the full maturation of ova indicated that if the temperature is low immediately after the blood meal is taken, a single feed is sufficient for full maturation, whilst if it is high, more than one feed is required. Thermal death points of the eggs, larvae, and pupae of *A. fluviatilis* were found to be 44°, 41° and 42°C respectively, the time of exposure being 5 minutes in each case.

Colonies of *A. fluviatilis* and *Aedes albopictus* have been kept up. The nocturnal activities of *A. stephensi* have been observed to commence much earlier than those of *A. fluviatilis*. A strain of *P. gallinaceum* has been maintained.

INSECTICIDE & MOSQUITO REPELLENT ENQUIRY.

The staff of this inquiry was established at Siliguri, Bengal, towards the end of August. A number of field investigations to test the value of various insecticides and repellents against the local mosquitoes, with special reference to *A. minimus* are in progress.

4. Statistical enquiry into the methods of forecasting epidemic malaria under Khan Bahadur Dr. M. Yacob, Assistant Director of Public Health, Punjab, and Mr. Satya Swaroop, Statistical Officer, Public Health Department, Punjab, Lahore.

Ever since 1922, the Punjab Public Health Department has been issuing, each year, two forecasts, namely, (i) the preliminary, prepared towards the end of August or the beginning of September and (ii) the final issued by the middle of September for indicating the probable degree of occurrence of epidemic malaria in different localities in the province. The method employed in the forecasts has so far been that developed by Colonel C. A. Gill, a former Director of Public Health, Punjab in his book " Genesis of Epidemics ".

A critical study has been made, from the statistical point of view, of the degree of accuracy attained in these forecasts. Although the statistical tests employed have been unavoidably stringent against the efficacy of the method of forecasting, yet, over the whole period of twenty years, viz. from 1923 to 1942, it has been established that the method enabled the officers of the department to specify in advance, with a significant degree of success, the localities where epidemic conditions subsequently developed. The experience of the twenty years has also been studied separately in two groups of ten years each. While in each of these two groups also the usefulness of the method has been statistically established, there has been no indication that the method has undergone any change in the degree of accuracy in the more recent years. The full results of this statistical investigation have been embodied in two papers namely, (i) " The forecasting of epidemic malaria in the Punjab ", Journal of the Malaria Institute of India, Vol. 5, June 1944, and (2) " Degree of accuracy realized in malaria forecasts in the Punjab for the years 1923 to 1942 ", Indian Medical Gazette, June 1944.

This preliminary investigation suggested that Gill's method of forecasting involved a personal factor in forming judgement about the probable incidence of malaria in any locality. It was considered that by the use of modern statistical methods his method could be freed from this personal factor and could thus be placed on a rigorous and statistically sound basis with perhaps greater chances of success.

Accordingly, the preliminary work of collecting and compiling basic data for a period of 77 years was undertaken. These data have been compiled on a monthly basis for the province as a whole and separately for 15 individual districts. During the process of compilation and charting it became evident that the annual epidemic figures of the province exhibited a periodicity of 8 or 9 years duration. The significance of this periodicity has been studied statistically and results have been embodied in a paper which is ready for publication. The main conclusions are that, in the Punjab, a significant decrease has occurred

over the period of 77 years (1867–1943) in the annual epidemic figures, thus demonstrating that, with the passage of time, an increasing degree of control has been exercised on epidemics breaking out in the province. The mathematical methods adopted for testing the existence of periodicity have their limitations in as much as they are designed to test periodicities of a fixed duration as in the case of physical phenomena. These methods strongly suggest the existence of an eight years cycle of malaria in the Punjab ; but in view of the period studied being not of a sufficiently long duration, and in view of the rigidity of the technique employed in testing it, the existence of the periodicity has not been conclusively established. For purposes of forecasting, however, it seems necessary that due notice should be taken in future of the possibility of the occurrence of a malaria epidemic after a period of 8 years. In some cases this period might fall short by one year and in others the appearance of the epidemic might be delayed by one year.

Some work has also been done on the assessment of the degree of relationship between the epidemic figure and rainfall in the case of Sialkot district. Gill postulated that the total rainfall in July and August was an important factor for forecasting. It has been found that whilst rainfall in August is highly correlated with the annual epidemic figure, the association is not statistically significant with the rainfall in July. Further work is in progress.

NUTRITION.

5. Nutrition researches under Dr. W. R. Aykroyd at the I. R. F. A. Nutrition Research Laboratories, Coonoor.

Work carried out by the Nutrition Research Laboratories has included the following :

The analysis of foods.

(1) Samples of yeasts, yeast extracts (marmite substitute), yeast brew, army vitamin tablets, wheat, ragi, rice, converted rice, barley and wheat flours, bread, biscuits, certain edible root flours, etc., have been analysed for their vitamin B_1 and nicotinic acid content. The enzyme method evolved last year in the laboratories was employed in these tests. The effect of washing and cooking on the thiamin and nicotinic acid content of a sample of converted rice obtained from the U.S.A. was studied. The results were similar to those obtained in this laboratory with parboiled rice.

(2) Extensive investigations have been carried out on "anti-thiamin" factor, which was found to be present, in certain cereals, and in certain oil seeds, viz. mustard, cotton and linseed. The factor was found to be composed of 2 components, one thermolabile and non-dialysable and the other thermostable and dialysable. Each component is active in destroying thiamin. The inactivation of thiamin was found to be non-enzymatic. Biological experiments carried out with rats and pigeons have shown that the factor rendered thiamin biologically inactive for these animals. In various respects the anti-thiamin factor under investigation differs from the anti-thiamin factor in carp, which behaves like a typical enzyme. Attempts are now being made to isolate (1) the factor itself, and (2) the break-down products of thiamin, formed by its action. The non-enzymic nature of the anti-thiamin factor suggests the possibility of certain metallic ions being involved in the inactivation of the vitamin. Preliminary experiments with mercuric chloride, silver nitrate, sodium pyrophosphate, sodium sulphite and potassium permanganate have shown that these salts inactivate thiamin.

(3) The pyridoxin (vitamin B_6) content of a number of foods has been determined, using the insect larvae technique. The values obtained agree fairly well with those obtained by Swaminathan's chemical method. Cereals and pulses are fairly good sources of this vitamin, while the foods usually designated "protective" are generally speaking poor sources. Dried yeast was found to be rich in pyridoxin.

(4) The nutritional requirements of 3 strains of lactic acid bacteria, viz., *Lactobacillus bulgaricus*, *Lactobacillus acidophillus* and *Streptococcus lactis*, were studied. All three strains were found to require riboflavin, pantothenic acid and biotin for primary growth. Using *L. bulgaricus* as the test organism, the pantothenic acid content of yeast, yeast extracts and various animal tissues has been determined. Most of the pantothenic acid in yeast and animal tissues is in the bound form and can be liberated by autolysis.

(5) Routine tests for vitamin A on a number of samples of shark liver oil have been carried out.

(6) Studies were undertaken on the stability of carotene in carotene concentrates of potency ranging from 25,000 to 80,000 I.U./g. and on margarine "fortified" with carotene. The potency of a carotene concentrate remained fairly unchanged during storage for 6 months in non-actinic bottles at Coonoor room temperature. Fortified margarines too did not lose potency during storage for similar periods, when stored in sealed tins. After opening the tins, a gradual destruction of carotene took place.

(7) The use of tapioca is rapidly spreading in South-West India. The nutritive value of this root is therefore a question of practical importance. A diet largely composed of tapioca, unless supplemented by some source of additional protein, fails to support the growth of rats. Analysis has shown that tapioca protein is not deficient in essential amino-acids ; quantitative and not qualitative deficiency is the important factor. The proportion of rice in a diet based on tapioca which is needed to produce nitrogenous equilibrium in rats was determined.

(8) Estimations of choline, methionine and cystine are being carried out.

(9) The investigation on soya bean was completed and the report submitted.

Animal experiments.

(a) Experiments with guinea-pigs have shown that vitamin P (hesperidin) accelerates growth on a diet containing sufficiency of vitamin C. A factor isolated from Bengal gram has a similar effect.

(b) Work on experimental fluorosis was continued. In experiments to assess the relative toxicity of 3 fluorides, sodium fluoride, calcium fluoride and magnesium fluoride, it was found that calcium fluoride was the least toxic, sodium fluoride being roughly 12 times and magnesium fluoride about 16 times as toxic as calcium fluoride.

Pandit and his co-workers concluded that vitamin C deficiency is a contributory factor in the causation of chronic fluorosis in man. The effect of vitamin C on fluorine poisoning in rats was investigated in rats in the Laboratories. Massive doses of pure vitamin C did not alter either the severity or incidence of fluorine poisoning in rats, rats receiving the vitamin having the same survival period as those not receiving vitamin C. These findings therefore fail to support the conclusions of Pandit and his colleagues.

(c) It is known that rats do not require vitamin C, being capable of synthesizing this vitamin. The mechanism whereby synthesis is achieved is unknown. It has been suggested that synthesis is effected by bacteria in the intestine. Sulfasuxidine which destroys intestinal flora, was given to rats fed on a diet complete except for vitamin C. No evidence of vitamin C deficiency has been detected in the animals.

(d) No evidence has been obtained that the resistance of rats to rat leprosy induced by the injection of infective tissue extracts can be modified by diet. Rats fed on the stock diet supplemented by (1) dried liver, and (2) dried brewer's yeast, at a 10 per cent level of intake developed rat-leprosy within the same period as rats in the same diet without supplements.

(e) *Choline metabolism :* Preliminary studies on the production of fatty liver on rats on low-protein, high-fat diets have been made. Egg albumin was found to be capable of producing fatty livers to a greater extent and in a shorter period than casein. Several fats and oils, commonly used in cooking, were

investigated for their comparative ability to produce fatty livers. It was observed that all the fats and oils tested produced fatty livers to almost the same extent.

(f) *Experiments with rice-moth larvae :* (i) Larvae, fed on a diet containing raw egg-white, develop "egg-white injury" or biotin deficiency, and die in about 15 days. When biotin is included in the diet, growth is resumed and increase in weight is proportional to the amount of biotin supplied. Avidin, the anti-biotin factor in egg-white, has the same effect as raw egg-white itself.

(ii) The synthesis of nicotinic acid by the rice moth larva has been observed.

(iii) It was found that when tryptophane was added to a pyridoxine-deficient diet, the larvae reared on this diet excreted yellow coloured faeces. The yellow colour disappeared on the addition of pyridoxine to the diet. Tryptophane added to a diet deficient in riboflavin, nicotinic acid, or pantothenic acid, did not give rise to the yellow-coloured larval excreta. Similarly, addition of pure amino-acids like cystine, tryosine, arginine, histidine, etc. to pyridoxine-deficient diet caused no appearance of yellow-coloured faeces. These results indicate that there is probably some relation between tryptophane and pyridoxine metabolism.

Clinical and field investigations.

(a) Investigations on "nutritional diarrhoea" have been carried out in the Stanley Hospital, Madras. After a good deal of preliminary sorting out of cases, a type of case has been identified which responds dramatically to the injection of nicotinic acid. Administration of the vitamin by mouth is less effective. Most cases show some degree of glossitis. Histamine-fast achlorydia is usually present and is not influenced by nicotinic acid. The mechanism by which nicotinic acid exerts its therapeutic effect is unknown. Nutritional diarrhoea of this type appears to be a common condition and is more frequently seen in females than in males. The patients are usually grossly malnourished. Fifty cases of nutritional diarrhoea have been analysed and successfully treated.

(b) A detailed statistical analysis has been made of the data obtained in the investigation of the relation between dental caries and fluorosis. This has confirmed the general conclusions previously reached. Study of the relationship between mottled enamel and dental caries in human beings is proceeding.

(c) Investigations on infantile beriberi in Cocanada are in progress. During the year the work has been hindered by the resignation of the Woman Medical Officer previously in charge. A new worker was appointed in July.

Nutrition Class.

A nutrition class consisting of 20 members was held from April 18th to June 10th. The class, which included a number of health officers deputed by governments, was a satisfactory one. Among the members were the newly-appointed Nutrition Officer in the Madras Public Health Department, health officers from the Punjab and Bihar, and a worker who has been appointed to organise a nutrition section in the War Services Exhibition.

Educational and advisory work.

Requests for information and advice were received from all over India. Numerous military nutrition problems were considered. Requests for information

and advice were received from Central and Provincial Food Departments, from other Government Departments, tea estates, missionary organizations, etc. The nutrition museum in the Laboratories, which is continually being extended, was visited by numerous people.

6. Ascorbic acid enquiry under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

BIO-SYNTHESIS OF ASCORBIC ACID UNDER THE INFLUENCE OF NARCOTICS.

In continuation of the researches on the increased daily urinary excretion of ascorbic acid (30-100 mg.%) by rats when fed with a variety of organic substances including narcotics, an attempt has been made to elucidate the mechanism of bio-synthesis from the study of :—

(1) General metabolism as reflected in urinary (a) sugar (b) phosphorus and (c) nitrogen.

(2) Blood sugar and liver glycogen under various conditions such as normal fed and fasted, or narcotised fed and fasted animals.

(3) Effect of detoxicating agent such as glycine.

(4) Part played by hormones such as insulin and adrenaline.

(5) Different dehydrogenase systems likely to be involved.

(6) Its dependence, if any, to other vitamins, specially of B-group.

1. Metabolic studies of about 50 male rats, normal and narcotised were made in 24-hours' urine for sugar, inorganic phosphorus and nitrogen. From the results it has been observed that a rat under narcotics is able to synthesise a considerably high quantity of ascorbic acid without any marked change in the excretion of urinary sugar, phosphorus and nitrogen from the normal. These results tend to suggest that this increased synthesis of ascorbic acid is not due to any glucogenic residue of 'extra protein' than that required for normal catabolism, nor was there any marked disturbance in phosphorylation of any component, if involved in the process.

2. Blood sugar and liver glycogen of about 40 male rats normal and narcotised have been studied under fed and fasted conditions. It has been observed that the excretion of ascorbic acid under narcotics remains practically unaltered by the change in the glycogen reserve of the rat to a minimum and rise of nitrogen and phosphorus to higher values during the progress of fast to 48 hours. It seems also doubtful whether this excretion of ascorbic acid results directly from the glycogen break-down. It also stands out from the nitrogen and phosphorus values that the excretion of ascorbic acid does not result from "extra protein" catabolism, nor was it affected by it.

3. Camphor, borneol etc. when fed to a living organism are detoxicated as conjugates of glucuronic acid and excreted in urine. These substances and a variety of narcotics when given to rats orally also stimulate the urinary excretion of ascorbic acid. With a view to ascertain whether increased ascorbic acid excretion results from some deficiency in the substances required for the detoxication mechanism of rats, glycine, which is known to be a good detoxicating agent and a fixative for aldehyde and keto products, was daily given to a number of rats under narcotics. No change in the enhanced excretion of ascorbic acid

by rats was, however, affected by daily supplement of glycine (20 mg.) nor could any ascorbic acid conjugate products be detected in the urine.

4. To study the role of hormones in the bio-synthesis of ascorbic acid by narcotised rats insulin and adrenaline were selected and not pituitous because it has been observed by others that the pituitary is not directly involved in the normal synthesis of ascorbic acid by rats.

For the study of the effect of insulin some male rats (120-150 gm.) were partially or fully depancreatised. From the weight gain and general state of well-being all these rats except a few appeared normal within 3 or 4 weeks of operation. The depancreatised rats were given daily intake of narcotics. So far as urinary excretion of ascorbic acid was concerned, these rats behaved like those normal but narcotised rats. That the excreted substance was ascorbic acid was further confirmed by biological assay. Control depancreatised rats without narcotics can excrete but slight traces of ascorbic acid in the urine. These results seem to indicate that insulin is not directly involved in the bio-synthesis of ascorbic acid by rats, at least that which is induced by narcotics.

For the study of the effect of adrenaline some rats (100–120 gm.) were adrenalectomised, most of which survived the operation and within 2-3 weeks began to gain in weight, though at a much retarded rate. Sufficient water and NaCl were supplied along with the diet. Care was also taken so that the operated rats were not exposed to great variation of temperature. Of the surviving adrenalectomised rats only 50% could stand the effect of daily intake of narcotics for more than a month. So far as urinary excretion of ascorbic acid is concerned these rats behaved like normal or depancreatised rats under narcotics. These results also indicate that adrenaline is not directly involved in the bio-synthesis of ascorbic acid by rats, at least that induced by narcotics.

5. Narcotics are known to have great effect on the different dehydrogenase systems. To study whether the bio-synthesis of ascorbic acid by rats under narcotics is the result of some change in or blocking of the enzyme centres, some dehydrogenases from brain and liver of normal and narcotised rats were studied with Thunberg methylene blue technique. Brain and liver were selected, because of all other organs, they are most likely to be affected by narcotics.

Citric, glutamic, glucose, glycerophosphoric, lactic, pyruvic and succinic dehydrogenases from brain and liver of about 50 normal and narcotised rats have been studied. Succinic and glycerophosphoric dehydrogenases of both brain and liver remains in normal and narcotised conditions, practically unchanged, so far as their activities are concerned. Others except pyruvic and lactic show, variable results from which on statistical analysis only, an inference can be made. It seems there is some inhibition of lactic and pyruvic dehydrogenase systems of the brain of narcotised rats. This observation along with that found in connection with B_1-deficiency seems to indicate that pyruvic acid system is in some way or other connected with the mechanism of the bio-synthesis of ascorbic acid.

6. Effects of Vitamin B_1-deficiency.

It has been observed here that the influence of narcotics in accelerating the bio-synthesis of ascorbic acid can be greatly suppressed when the rats are B_1-deficient. Other vitamins of B-group seem to play no part. From the results so far obtained vitamin B_1 seems to be involved in the synthesis of ascorbic acid by rats.

7. Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

Relationship of hypoproteinemia and oedema.

The investigation made in the year 1942 "on the relationship of hypoproteinemia and oedema" was repeated because it was felt that the first effort was fragmentary and that a complete survey could be accomplished with the large number of cases of purely nutritional oedema that are now being admitted to the Hospitals.

Starling in 1896 first suggested the relationship of intracapillary pressure tending to force fluid through the wall of the capillary and the osmotic pressure of the blood plasma attracting fluid back into the capillary. Then many cases of oedema were recognised as due to malnutrition during the Great War but the mechanism of its production by hypoproteinemia was not realised. Epstein in 1917 first suspected that the lowered osmotic pressure of hypoproteinemia as the cause of oedema of nephrosis. Others soon applied the idea in the case of nutritional oedema and proved low plasma proteins in such cases. Moore and Van Slyke assert that the critical level of the appearance of oedema is 5.5 gram of total protein and 2.5 gram of serum albumin. Other factors such as high salt and fluid intake will precipitate or increase oedema if both are available. These secondary factors often belie the so-called critical level.

Twenty-five cases of nutritional oedema were studied during the last 4 months; 12 in children, 10 in adult males and 3 in pregnant women. The clinical criteria of deficiency and malnutrition were those generally accepted. Records were kept of the duration of disease, salt, and fluid intake. Other complications such as anaemia or diarrhoea if present were also noted. Determination of plasma proteins were carried out on admission and during treatment. A few results are given in table 1.

Table I.

Plasma protein in patients of nutritional oedema, on admission.

No. of cases in.		Total protein.	Albumin.	Globulin.	Fibrinogen.
In children	1.	4.06	1.17	2.88	0.40
	2.	4.75	2.02	2.73	0.29
	3.	4.56	2.01	2.55	0.12
	4.	4.15	1.65	2.50	0.21
	5.	5.30	2.45	2.85	0.27
In adult males	1.	4.16	1.68	2.48	0.17
	2.	4.50	1.50	3.00	0.43
	3.	4.08	1.80	2.30	0.26
	4.	4.25	1.50	2.75	0.43
In pregnant women	1.	4.94	2.71	2.14	..

These results clearly indicate that the level of plasma protein at which oedema appeared in these patients were much below the critical level 5.5% ; and albumin level 2.5%. The globulin in most cases were within normal limits.

Some of the patients improved on high protein diet. With clinical improvement, the level of plasma protein also increased—the increase being due to an increase in the albumin fraction. A typical result is given in table II.

Table II.

Date.	Total protein.	Albumin.	Globulin.	cell vol.	oedema.
14/11/44 on admission	4.33	1.90	2.43	28%	++++++
29/11/44	5.48	2.86	2.62	32%	nil

Two patients who did not respond well to high protein diet alone showed considerable improvement with vitamin B_1 along with high protein diet.

Those patients who proved resistant to treatment and who did not improve clinically showed no change in the concentration of plasma proteins and will form the subject of further investigation.

The influence of protein deficiency on the gastrointestinal tract has specifically been studied. Macrey, Barden and Radvin (1937) demonstrated that hypoproteinemia is associated with pronounced retardation of the passage of water-barium sulphate meal through the small intestine of the dog. They attribute this to the oedema of the small intestine. These observations suggest an impairment in intestinal absorption in hypoproteinemia and oedema.

Nitrogen balance studies, liver function tests and also the determination of time of passage of barium meal through the intestine are being carried out to determine whether impairment of intestinal absorption due to chronic malnutrition or impaired hepatic function is associated with the lowered plasma protein and the nutritional oedema in these patients. Very little clinical study has been made from this point of view. In view of the great abundance of clinical material available, these studies would form a suitable subject for further investigation.

Hyperproteinemia and hyperglobulinemia.

Hyperproteinemia may not be as rare an occurrence as was once believed. An increase in the concentration of total protein is invariably due to an increase in globulin. Hence "a study of hyperproteinemia is essentially a study of hyperglobulinemia."

In 1937 Jeghers and co-workers reviewed the subject and presented 13 cases of hyperproteinemia. Cardon and Aston (1943) presented 54 cases in which total protein was above 8.5 gram %. Kagan (1943) reported 50 cases due to 16 different causes.

There is little information available regarding the general incidence of hyperproteinemia, although it is likely to vary in different parts of the world. In localities where kala-azar and lymphogranuloma is prevalent, the incidence of hyperproteinemia may be more common.

The present report deals with 50 cases of hyperglobulinemia associated with hyperproteinemia.

Diseases involving bone marrow : In a case of monocytic leukemia reported by Kagan, the total protein was 10.1 gram %, the globulin varying from 4.3 to 6.7. Since monocytes are considered as a part of the reticulo-endothelial system, he suggests that should such marked increase of serum globulin be common in

this disease, it would lend further support to the view of Bing and Plum that *globulin is formed* by the reticulo-endothelial system.

The present series includes 1 case of leukemia, in which the total protein was 8.75, the globulin 5.68. The increase of globulin was due to an increase in pseudoglobulin fraction.

Infection. Hyperproteinemia have been reported in various chronic infection. Most of the cases reported in this series are due to chronic infection.

(1) *Kala-azar.* The highest value of serum protein in this series was 11.2 gram % in a case of kala-azar, the globulin being 9.06%. The albumin was only 2.27%, since infection interferes with the formation of albumin. When the source of infection was removed after treatment, albumin returned practically to normal value. The globulin still remaining at a higher level 5.45%. Hence the determination of albumin is of considerable prognostic value in such cases.

(2) *Tuberculosis.* Eichelberger (1937) studied plasma protein in 109 patients with tuberculosis. The result revealed that hyperproteinemia was, present in 44% of cases, the globulin above 3% in 62 % of cases, and the albumin below 4% in only 17% of cases. *In none of the cases was the total protein below normal.* Kagan however observed hypoalbuminemia and decreased total protein with greater frequency in 50 cases studied by him.

In the present study, the total protein above 8 gram % was observed only in 8 cases out of 50 cases studied. The globulin above 3 gram % in almost all cases and above 4.5% in 11 cases. Inspite of considerable increase in globulin, the total protein much below normal was found in 16 cases and albumin below 3 gram % in 17 cases. The infection is no doubt responsible for the increase in globulin, but the low albumin resulting in decreased total protein is due to the malnutrition which is so common in this country. The value of albumin is of considerable importance since this indicates the nutrition of the patients.

(3) *Syphilis.* Rowe (1916) stated that in syphilis globulin is definitely increased. Lloyd and Paul (1926) studied 11 cases of syphilis. In eight cases the globulin was above 3 gram %. In only 1 case was the total protein above 8 gram %. Recently the occurrence of hyperproteinemia and hyperglobulinemia in uncomplicated syphilis has been questioned by Jones and Rome (1936), which according to them is due to the concomitant presence of lymphogranuloma venereum.

In the present study, the total protein was above 8 gram % in 13 out of 22 cases. The increase in total protein was due to an increase in globulin. The albumin was above 4% in 14 cases.

In 3 cases of lymphogranuloma, recently studied, the total protein was above 9 gram % in all cases, the globulin above 5.6%, and the albumin below 3% in 2 cases. From the result of several cases reported by Cardon and also from the result of three cases studied by us, it would appear that the change in the different fraction of the blood plasma may be different in the two diseases. Further study to find out the incidence of lymphogranuloma in this country and the extent to which syphilis is complicated by this disease is in progress.

(4) *Liver diseases.* Hyperglobulinemia have been reported in some type of liver disease, especially cirrhosis. Of the 9 cases of hepatic disease reported by Cardon (1943), there are 6 cases of cirrhosis. The total protein ranging from 8.0 to 13 gram %.

Hyperglobulinemia is found in most cases of liver disease ; the most significant increase being in the euglobulin fraction. Inspite of considerable increase in the globulin fraction the total protein was below normal in 50% of the cases studied in the present investigation. The present series includes only 6 cases of hyperproteinemia out of a total of 40 cases studied recently. No reason can be ascribed for the hyperproteinemia in certain types of cirrhosis.

(5) *Acute catarrhal jaundice.* There were three cases of acute catarrhal jaundice with hyperproteinemia. The increase in the concentration of the total protein was determined by increase in globulin, the albumin remaining normal; thus differing from those of cirrhosis.

(6) *Glomerulonephritis.* Kagan reported that hyperglobulinemia was seen in 50% of determinations done in patients with acute glomerulonephritis. An increase in the concentration of globulin above 3.5 % has been observed in most of the cases studied by us, due to the associated infection, but hyperglobulinemia associated with hyperproteinemia in nephritis is rare. Only 6 cases of hyperproteinemia have been observed out of a total of 60 cases studied.

(7) *Miscellaneous conditions.* In the miscellaneous group in this series are cases of malaria with total protein above 9%, 1 case of lung abcess ; 1 case of psoas abcess, a few cases of undiagnosed fever and 1 case clearly due to dehydration of vomitting.

Hyperglobulinemia of greater or lesser extent occurs in most infectious diseases. Little is known about the exact nature of the increase in globulin in chronic infections. The present study include results of an extensive series of determination of different globulin fraction in different diseases. Aside from its role as antibody, almost nothing is known of the function of globulin. Kagan suggests that if the entire increase of globulin is due to active antibodies, the body is thereby protected against such prolonged and serious infections. Perhaps much of the serum globulin is not active antibody.

An investigation on cases of hyperglobulinemia associated with hyperproteinemia may throw more light on the source of globulin. Fractionation of globulin in different diseases may lead to the significance of each fraction and to the nature of complications in different diseases.

8. Enquiry on the role of manganese in the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

These investigations are in continuation of those reported last year.

Young guineapigs kept on a scorbutic diet were divided into 3 groups. Group 1 received ½ c.c. (25 mg.) of glucose injection daily. Group 2 received 1 c.c. of 0.04 % of manganese solution daily. Group 3 received 25 mg. glucose in ½ c.c. of 0.04 % manganese solution. The experiment lasted for 14 days. The pigs were then killed and the histological structure of the root of the incisors studied. Both groups II & III showed an average protection much higher than Group I which had no protection. This conclusively proves that manganese is definitely required by animals for synthesis of ascorbic acid.

An investigation on the retention of manganese by rats, on diets supplemented with sodium bicarbonate and ammonium chloride was started but had to be abandoned as the enquiry was terminated. However it has been found that there is more excretion of manganese by a group of rats whose guts have been made acid with ammonium chloride.

9. Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.

Earlier work under this enquiry on the composition of the diets in the Kangra Valley and on the investigation of calcium, phosphorus and phosphatase of the blood serum, indicated the gross deficiency of calcium and relatively high proportion of phosphorus in the diet, and the consequent depression of calcium in the blood to a fairly severe degree particularly in women; value of calcium ranged between 6.40–7.84 mgs/100 c.c. of blood serum. The observations indicated a pathological condition of high phosphorus and low calcium type, though the clinical symptoms were similar to those obtained in individuals living on low phosphorus and high calcium diets, met with in other parts of the world.

During this year a similar investigation, in the poorer quarters in the city of Lahore, has been followed. The dietary ratio of calcium : phosphorus ranged between 0.5–0.6. The blood values are on the lower limits of the normal range. No pathological condition has been observed in this group of population.

A similar investigation was undertaken in the Kulu valley, a neighbouring valley to Kangra, during this summer. Blood samples from 68 cases including males, females, children and 15 cases of goiter were examined. Blood serum calcium was above 10 mgs/100 c.c. in all cases, excepting in those of goiter, in which a somewhat lower value with a mean of 9.75 was obtained. Values for blood serum phosphorus ranged between 3.44–3.76 mgs/100 c.c., while in cases of goiter these were slightly elevated (mean 4.28 mgs/100 c.c.). Blood phosphatase ranged between 1.45 and 2.92 units for adults and 7.7 to 8.05 units for children. These are within normal range. Thus in this neighbouring area both the blood picture and the clinical condition are normal. The diet of this area is being examined for their mineral and their growth promoting properties.

This comparative study of diets, blood picture and clinical conditions of the population in two neighbouring areas when completed, will be of value in assessing the causes which lead to a high abnormality of mineral metabolisms in one of the areas.

Last year the scope of this enquiry was extended to include the examination of different forms of phosphorus present in the blood of normal individuals and pathological cases. These included preformed inorganic phosphorus, adenosinetriphosphate, hexosediphosphate, hexosemonophosphate, acid soluble phosphorus, glycerophosphate, lipid and nucleic acid phosphorus and total phosphorus. This study is also pursued this year in the hope of its leading to some interesting results, but at the present moment there is nothing of any significance to report.

Six papers containing the results of the investigations have been communicated for publication to the Indian Journal of Medical Research.

10. Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipur, Patna.

Human feeding reports with the different ratio of rice & pulse as also rice & milk protein.

Introduction.

In poor Indian homes proteins from pulses and cereals supply the major source of nitrogen in the diet. In the present investigation an attempt has been made to find out whether rice protein : pulse protein ratio makes any difference as to the biological value of the mixed proteins. Subsequently in a similar series of experiments pulse proteins in the test diets were completely replaced by milk proteins.

Rice and pulse protein.

Feeding experiments were conducted on six healthy adult human males weighing from 40 to 58 kg. The test diet consisted of medium quality parboiled rice and red gram (Cajanus indicus) in such proportions that at the three levels of feeding, the ratio of rice protein: pulse protein was 100:75, 100:100 and 100:125 respectively. The rice and pulse were cooked separately. The vegetable quota in diet was kept constant and consisted of 120 g. of potato, 150 g. of bottle gourd and 200 g. of leafy vegetable, cooked in vegetable oil. The quantity of rice varied from 360 g. to 630 g. and that of pulse from 100 g. to 175 g. in the different test diets with the different subjects. Small quantities of salt and spices were added to suit the taste. These diets simulated the meals ordinarily consumed by the subjects in their own homes. The proteins from the vegetables never exceeded 15% of the total protein in the food. The total amount of protein in all the diets were almost constant for each subject and the level of protein intake was between 9 and 10 per cent. Every subject was on each test diet for a period of one week and the faeces and urine were collected during the last three days of the period. The calorie value of the diets varied from 2800 to 3500 daily. The subjects performed their normal duties during the test period.

Rice and milk protein.

The above feeding experiments were repeated on the same subjects. In these later series the pulse quota in the test diet was replaced by fresh cow's milk in amounts necessary to replace the pulse protein by an equivalent amount of milk protein. The ratio of the rice protein : milk protein at the three different levels of feeding employed were 100:75, 100:100 and 100:125 respectively as in the case of pulse red gram. The daily intake of milk varied from 700 c.c. to 1400 c.c. in the different experiments.

Biological value.

The average biological values of each of the test diets are enumerated below :—

Proportion of proteins.	*Biological value.*
Rice protein 100 : Pulse protein 75	57.8
Rice protein 100 : Pulse protein 100	59.2
Rice protein 100 : Pulse protein 125	56.4
Rice protein 100 : Milk protein 75	67.8
Rice protein 100 : Milk protein 100	65.5
Rice protein 100 : Milk protein 125	65.5

No difference could be noticed statistically between each of the three levels of feeding within the pulse group or within the milk group. But mixture of rice and milk proteins were found superior to the mixture of rice and pulse proteins.

FEEDING EXPERIMENTS WITH MIXTURES OF CEREALS.

Fresh feeding experiments have been started on two human subjects in order to find out the nitrogen balance of a mixture of cereals in a poor man's diet. The pulse and vegetable quota has been kept constant but a mixture of rice and wheat, rice wheat and maize, rice wheat and barley, rice wheat and millet has been given and the daily intake and excretion of nitrogen is being determined. The feeding experiment has just been started and is being continued.

11. Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore.

The oil extraction centres on the East and West coasts of Madras Presidency were visited to examine critically the methods of manufacture with a view to find out and rectify any defects which might start the development of rancidity. The observations have been summarised below :—

The average marketed sample of shark liver oil has a high acid value which was shown to be produced during the storage of livers after removal from the sharks, and before the extraction of their oil.

STORAGE OF LIVERS.

A number of livers were cut in the middle at the junction of the lobes and the corresponding halves mixed to form two batches. The oil from one batch was immediately extracted by boiling in water, the other batch being preserved in 20% common salt for a definite interval before treatment. The examination of the oils from the fresh and preserved livers from a series of such experiments where the livers were preserved for increasing periods of time showed, that during storage (1) the acid value progressively increased, (2) the yield decreased, (3) the colour deepened and (4) an unpleasant odour developed.

Preliminary efficient mincing of the livers before mixing with salt retarded considerably, but did not inhibit the rate of development of these undesirable changes. The vitamin A content of the oils was not affected in either case.

INFLUENCE OF ACIDITY ON THE STABILITY OF THE OIL.

The total fatty acids in an oil were isolated by saponification, and added to the same sample of oil thus increasing the acidity. The results of stability

tests with the control and acidified samples of oil showed, that free fatty acids in shark liver oil accelerate the development of rancidity, and the destruction of vitamin A. This emphasises further the importance of using only fresh livers for oil extraction when an oil of very low acid value can be obtained.

INFLUENCE OF MOISTURE ON THE STABILITY.

Moisture was found to be inert, since no difference was observed in the rate of oxidation of the glycerides and of vitamin A when the oil was dehydrated with anhydrous sodium sulphate, or water (1—2%) was added to it. The increase in the peroxide number and the amount of vitamin A destroyed in the dehydrated oil, moist oil (0.2 to 0.25% volatile matter and water) and oil containing over 2% water were of the same order after storage for twenty days.

INFLUENCE OF LIVER DEBRIS ON THE STABILITY.

Generally the crude oil contains small amounts of suspended cooked liver particles which remain in the oil for an appreciable length of time before filtering. The influence of these particles on the keeping quality of the oil was studied because of the practical importance of the problem. The increase in free fatty acids and the extent of destruction of vitamin A in the oil containing the cooked liver particles were of the same order as in the filtered oil free from the impurities, after storing for thirty days. However, the former developed a foul odour and dark colour.

INFLUENCE OF GROUNDNUT OIL ON THE STABILITY.

In view of the extensive use of groundnut oil as a diluent, the influence of this on the stability of shark liver oil and the vitamin was studied by accelerated oxidation tests. When shark liver oil was mixed with freshly refined, good-quality of groundnut oil the induction period was considerably prolonged—it was rendered more stable to oxidative rancidity. The quality of the diluent oil is an important criterion, since one sample of stored oil actually promoted oxidation.

However, it is felt that the practice of adulteration with groundnut oil should be discouraged. For getting an oil of uniform potency it is suggested, that oils containing different amounts of vitamin A should be judiciously blended.

RECOVERY OF OIL FROM WASTE LIVER DEBRIS.

In view of the shortage of shark liver oil the possibility of increasing production by pressing the liver residue after cooking was investigated. Provided the liver debris was pressed immediately after cooking or kept hot till the pressing, the increase in the acid value of the resulting oil was very little. The vitamin A content of the pressed oil was undiminished; and the only disadvantage was an appreciable darkening of the colour.

ANTIOXIDANTS.

The following substances were found to be ineffective as antioxidants in shark liver oil—oxalic, tartaric, malonic, maleic, citric, orthophosphoric, succinic and glutamic acids, and glycine.

12. Enquiry into the relationship between vitamin B complex and tumour growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

This enquiry was undertaken towards the middle of 1943 as there were indications in the literature that members of the vitamin B complex influence

the growth of tumours in animals and in man. The published reports from the different laboratories abroad were conflicting. It was therefore decided to undertake a systematic though laborious study of this problem. The work was undertaken along three different lines as stated below. —

1. Determination of the normal content of nicotinic acid and riboflavin in strains of mice susceptible and insusceptible to mammary cancer.

2. Vitamin B content determination of livers during the course of development of hepatic cancer following continued ingestion of butter yellow in albino rats.

3. A study of the urinary excretion of certain members of vitamin B complex and their saturation in cancer patients.

Laboratory findings in exerimental animals.

1. During the course of the year three inbred strains of mice having a varying susceptibility to mammary cancer were studied. (a) C_{57} Black, a relatively insusceptible strain with an incidence of spontaneous breast cancer of about 1%, (b) Strains C_3H and A who develop spontaneous cancer of the breast in about 80 to 95 per cent of adult females. The breeding as well as non-breeding females of C_3H strain are liable to spontaneous disease to the extent of 95 %, while in A strain the virgin females show an incidence of only 4.5 % and the breeding females develop breast cancer in 80 to 85 % of their population. Data have been collected as regards the nicotinic acid, riboflavin, ascorbic acid and glutathione content of the livers of these three strains of mice and discussed in a paper which has been prepared for publication. The results are summarised in table I.

Table I.

Mean liver contents of the three strains of mice.

Strain	Nicotinic Acid ug/gm		Riboflavin ug/gm		Ascorbic Acid mgms. per cent		Glutathione mgms. per cent reduced		Total	
	M(8)	F(12)	M(4)	F(6)	M(8)	F(12)	M(8)	F(12)	M(8)	F(12)
C 57	103.7	107.9	33.7	35.3	25.2	18.6	232.9	220.7	249.8	225.0
C_3H	112.6	119.2	34.3	36.6	26.3	24.9	283.9	214.4	307.0	225.8
A	115.3	129.5	34.6	37.1	35.8	29.5	308.3	293.1	311.3	308.9

Notes: (i) Numbers in the brackets refer to the number of mice.

(ii) M — Male ; F — Female.

(iii) Significant differences of the liver contents between the sexes and the strains have been shown by lines with the following conventions :—

(a) The arrows point at the higher of the two values.

(b) Chance less than 1 in 20 and more than 1 in 100.

Chance less than 1 in 100 and more than 1 in 1000.

Chance less than 1 in 1000.

2. As it was realised quite early, that the means at the disposal of the laboratory would not permit the simultaneous investigation of all the members of vitamin B complex it was decided to commence this part of the work with a study of nicotinic acid and riboflavin content of livers. 96 animals (albino rats) were used for the experiment. They were divided into eight different groups each consisting of eight experimental and four control animals. The experimental animals were fed 0.4 to 0.5 ccs. of olive oil containing 8 to 10 mgms. of butter yellow by a stomach tube on alternate days while the controls were fed 0.4 to 0.5 ccs. of olive oil only. The intermediate days were included as days of feeding in the final computation. These different groups of rats were sacrificed at intervals of usually about one month after the first 45 days, till 360 days of butter yellow feeding. After a period of 300 days feeding one female of the experimental group developed a hepatoma. In the group sacrificed after 360 days of butter yellow feeding only three females out of the experimental group consisting of four males and four females showed large masses of hepatic tumours. The results of analysis of the nicotinic acid and riboflavin content of livers of rats sacrificed at successive periods of butter yellow feeding and of their corresponding controls are summarised in table II.

Table II.

Group of Albino rats.	Days of butter-yellow feeding.	Animals used in the experiment.	Nicotinic Acid ug. per gm. of fresh tissue	Riboflavin ug. per gm. of fresh tissue.
1	45	E. 4 males & 4 females	62.1 ± 7.1	103.2 ± 10.9
		C. 2 „ & 2 „	86.4 ±10.6	110.6 ± 8.6
2	90	E. 4 males & 4 females	120.1 ± 3.2	105.2 ± 7.0
		C. 2 „ & 2 „	131.0 ± 2.6	86.8 ± 4.6
3	120	E. 4 males & 4 females	81.3 ± 6.7	85.0 ± 8.5
		C. 2 „ & 2 „	141.6 ± 4.8	63.2 ± 5.3
4	150	E. 4 males & 4 females	120.3 ± 6.7	76.9 ± 2.9
		C. 2 „ & 2 „	139.7 ± 5.4	76.6 ± 10.4
5	180	E. 4 males & 4 females	122.7 ± 8.3	113.7 ± 11.2
		C. 2 „ & 2 „	131.7 ± 7.4	81.3 ± 3.3
6	240	E. 4 males & 4 females	143.5 ± 7.9	91.1 ± 4.5
		C. 2 „ & 2 „	151.2 ± 5.8	112.4 ± 4.4
7	300	E. 4 males & 4 females**	137.4 ± 1.6	104.6 ± 5.3
		C. 2 „ & 2 „	134.4 ± 9.8	93.6 ± 12.1
8	360	E. 3 males & 4 females**	85.9 ± 2.2	28.9 ± 3.4
		C. 1 male & 2 females	132.2 ± 6.9	96.4 ± 13.6

**One female in group 7 and three females in group 8 developed tumours of the liver.
E—Experimental animals.
C—Control animals.

It would be seen that after butter yellow feeding for 120 days there is a sharp drop in the nicotinic acid content of livers of the experimental animals. This period corresponds to the stage reported in the literature when an anaplastic proliferation of tumour cells takes place. In the present experiment, however, it was found that these rats were able to overcome this change and adjust their metabolism to the ingestion of butter yellow without the development of any visible morbid changes in the liver. Another drop in nicotinic acid content of livers occurred in the group of rats sacrificed after 360 days of feeding. It was interesting to note that in this group three females of the experimental animals developed large masses of hepatic tumours.

A fall in riboflavin content of liver also occurred in the groups—the experimental as well as the control animals—sacrificed after 120 and 150 days of butter yellow feeding. This change could not, therefore, be attributed to the action of butter yellow. A second drop which may probably be associated with tumour development was noticed in the group sacrificed after 360 days of butter yellow feeding.

3. Clinical investigations on hospital patients: Metabolism studies on nicotinic acid were carried out in 24 patients having oral cancer (confirmed by the histological examination). Table III shows a comparison of the urinary excretion of nicotonic acid and trigonelline in normal subjects and in patients with oral cancer. The urinary excretion of nicotinic acid and trigonelline in the two groups did not show a significant difference. It was therefore thought advisable to determine the condition of saturation with test dose technique in these two groups. Ten patients out of these 24 were administered orally a test dose of 250 mgms. of nicotinic acid. The urine excreted during the next 24 hours was examined and the total quantity of nicotinic acid and its derivatives determined. A second test dose was administered the next day and the urinary excretion was similarly studied. The maximum amount of nicotinic acid recovered in the urine of normal subjects after the second test dose was 18.93%. This proportion agrees with the findings of Sarett *et al* in normal people. In the case of cancer patients however it was only 8.02%. The minimum recoveries were 7.54% and 0.7% in the normal and in the cancer patients respectively.

Table III.

A COMPARISON OF THE EXCRETION OF NICOTINIC ACID AND TRIGONELLINE IN TEN NORMAL SUBJECTS AND TEN PATIENTS WITH ORAL CANCER.

	Before test dose.	After 1st test dose of 250 mgms. of nicotinic acid.		After 2nd test dose of 250 mgms. of nicotinic acid.	
	*Urinary excretion in 24 hrs.	Urinary excretion in 24 hrs.	*Per cent *recovery	Urinary excretion in 24 hrs.	Per cent recovery
Normals ..	20.09	45.38	11.22	56.67	15.04
Standard Error ..	± 2.72	± 6.70	± 1.62	± 4.91	± 2.00
Oral Cancer ..	13.94	18.87	2.33	22.65	3.24
Standard Error ..	± 2.36	± 2.59	± 0.43	± 2.39	± 0.79

* Urinary excretion refers to the total excretion of nicotinic acid and trigonelline calculated in terms of nicotinic acid.

* Per cent recovery implies the proportion of nicotinic acid and trigonelline to the test dose given, recovered in urine after deducting the amount excreted previous to the test dose.

ASSESSMENT OF RESULTS.

The investigations summarised above have been instructive in the following respects :

1. A study of literature on the subject showed that p. dimethyl aminoazobenzene (butter yellow) is a potent carcinogenic chemical which produces liver cancer in different strains of albino rats. The time of appearance of tumour appears to vary with different strains of rats and with the diets used (Table IV).

Table IV.

	Carcinogen gms. %.	Strain.	1st appearance of tumour, approx. days.	Tumour Incidence* %	Diet**
Sasaki (1935)	o. aminoazotoluence 0.10 gms. %	Albino rats	195	100% between 8.5–10 Months	A
Kinosita (1937)	p.dimethylaminoazobenzene 0.06 gms. %	Albino rats of mixed strain	50	100% after 5 Months	A_2
Emmart (1940)	o.aminoazotoluene 0.15 gms. %	Wistar, Buffalo, and Osborne Mendel	210	88% between 10–20 Months 100% after 15 Months	C
Edwards (1941)	p. dimethylaminoazobenzene 0.06 gms. %	Osborne-Mendel	100	60% after 9 Months	D
Orr (1940)	do.	Albino rats	90	60% between 5-7 Months	A & B
Sugiura (1941)	do.	Wistar	51	100% after 3 Months	A_1
	do.	Sherman	48	100% after 2 Months	
	do.	Evans	80	85% after 5 Months	
Miller (1941)	do.	Stock Albino rats	120	90–100% after 3 Months	E

* Tumour incidence at a given time is the percentage of tumour bearing rats to the number of rats surviving at that time.

** Diets :

A — Unpolished rice, green vegetables plus adequate water.

A_1 — Unpolished rice, carrot and adequate water.

A_2 — Unpolished rice, green vegetables, dried sardines and adequate water.

B — Equal parts of broken-up maize, wheat and oats, green vegetables and water.

C — 15% whole milk powder, 75.47% ground wheat, 4% dried yeast, 4% codliver oil, 1.47% sodium chloride, 0.13% ferric chloride and adequate water.

D — Casein 6%, sucrose 15%, starch 50%, lard 25%, Osborne Mendel salt mixture 4% and 400 mg. yeast and 100 mg. codliver oil per rat per day.

E — Crude casein 9–12%, cerelose 80–77%, salts 4%, cotton seed oil 5%, codliver oil 2%, vitab—0.2 gms. or 4 drops per rat per day.

The basal diet used in most of the experiments consisted of rice, carrot or green vegetables and an adequate supply of drinking water. It was pointed out by Miller, that the rice carrot diet was inadequate in many respects for the nutritional requirements of rats as evidenced by the very poor growth even in absence of the carcinogene, a fall in body weight after the feeding of butter yellow and a high mortality during the course of experiment. The diet suggested by Miller though relatively low in proteins (9 to 12 %) was reported by him to be adequate for normal growth and general health of the experimental animals. The tumour incidence on this diet after four months of feeding with butter yellow was 90 to 100 %. This incidence did not differ much from that reported by Nakahara, Sugiura, Orr, and Kinosita all of whom used the rice carrot diet.

In the present investigation the diet recommended by Miller was therefore adopted and was suitably modified owing to an inability to obtain substances used by him in his experiments. In the course of the experiment no tumours developed after four months of feeding the dye. The animals were healthy and the body weight increased steadily. Contrary to the experience of Miller it was only after 12 months of feeding that 3 females out of a batch of 8 experimental animals showed large masses of hepatic cancer.

2. The rats showed a high mortality even before the development of tumour in the experiments reported by other workers (Kinosita, Nakahara—60 to 90 %, Sugiura 27%, Miller 30 to 50%). In the present experiment only one animal died after 240 days of feeding the carcinogen and showed an acute bronchopneumonic condition. No gross change was observed in the liver.

3. The first fall in the nicotinic acid content of liver occurred at 4 months of feeding butter yellow and a second drop both in nicotinic acid and riboflavin occurred subsequent to the actual appearance of tumours after 360 days of feeding.

4. Since no tumours appeared till after 10 months of feeding it was likely that the precancerous period accompanied by necrosis, cirrhosis etc. was very short.

5. The experimental data referred to above suggest that the delay in the appearance of hepatic tumours in rats following butter yellow ingestion may be attributed either to (a) an unusual behaviour of the local strain of albino rats showing a marked insusceptibility to the development of such tumours, or (b) the protective action of casein along with the crude rice protein and an adequate amount of riboflavin (120 ug/rat/day) in the diet used for this investigation, or (c) a combination of the above two factors.

Proposed plan for future work.

It was unfortunate that the groups of experimental animals were finished at a most interesting phase of the investigation. It is obvious that it is necessary to repeat the present experiment and to design it in such a way that the animals could be sacrificed at periods ranging from 300 to 500 days of butter yellow feeding. An extension of the experiment along the following lines would also be desirable.

1. A repetition of the experiments of Kinosita and of Sugiura by using rice and carrot as the basal ration and a study of the nicotinic acid and riboflavin contents of livers in such animals.

2. A study of the effects of the damage to the liver parenchyma by toxic substances during the butter yellow feeding of the rats.

3. A study of the incidence of liver cancer induced by butter yellow on a diet suggested by Himsworth and reported by him as producing chronic continued damage to the liver parenchyma of the experimental rats.

4. Studies on metabolism of other members of vitamin B complex in patients suffering from oral cancer. It might be mentioned that the main reason for the choice of patients suffering from oral cancer for these investigation is due to (a) a high incidence of oral cancer in patients admitted for malignant disease at the Tata Memorial Hospital and (b) to the fact that Martin and Koop have associated the presence of precancerous lesions in the mouth with avitaminosis B, on the basis of a large clinical experience.

13. Enquiries into (1) tests of the biological value of soya bean protein (2) growth tests with soya bean and (3) human experiments with soya bean under Dr. Bashir Ahmad, at the Punjab University Institute of Chemistry, Lahore.

During the year work was continued on the effect of heat treatment on the biological value of the protein of Soya Bean and other pulses, and on the investigation of the limiting factors in a poor rice diet by further supplementation of this diet with calcium, vitamin A, vitamin B complex in addition to soya bean and pulses. There is a definite improvement of the biological value of the proteins of soya bean and pulses on autoclaving and parching. At 10% level of protein intake the biological value of soya bean by the growth method increased from 0.78 to 1.1 on baking as reported before. Similarly the biological value of Bengal gram by the growth method increased from 1.33 to 1.77 after autoclaving and from 1.33 to 1.83 on parching. The biological value of green gram increased from 0.95 to 1.04.

The poor rice diet which has a composition of 6.25% protein, 1.28% ash, 0.027% calcium, 0.102% phosphorus, and a calcium phosphorus ratio of 1 : 4 showed poor nutritive value and growth promoting properties. The value for the basal diet was found to be 0.26. On supplementing it with soya bean it rose to 0.82 and on supplementing with Bengal gram 1.1.

The effect of further supplementing of this diet with calcium and vitamins have been studied by the addition of yeast extract and calcium lactate and the following results have been obtained :—

1. Basal diet+Yeast extract+Calcium lactate	.. 7.9
2. Basal diet+Yeast extract+Calcium lactate+Soya bean	.. 11.1
3. Basal diet+Yeast extract+Calcium lactate+Bengal gram	.. 12.0

Further work along these lines is in progress.

14. Nutrition research unit under Dr. K. P. Basu at Dacca University, Dacca.

Metabolism of fat.

(1) *Rate of absorption of various fats and oils.* Investigations on the rate of absorption of various fats and oils started in the previous year, have been continued with more oils. For this purpose 2 c.c. of the melted fat is fed to a rat by means of a stomach-tube and the portion of it remaining in the stomach and the intestine after a definite interval is found out. The following are the main results :

Names of oil or fat	Percentage absorbed in		
	2 hrs.	4 hrs.	6 hrs.
Cow-butter fat ..	42	48	59
Coconut oil ..	36	43	49
Olive oil ..	41	49	61
Mustard oil ..	28	56.3	64

(2) *Inter-relationship between fat and calcium metabolism.* Investigations on the interrelationship between fat and calcium metabolism is being continued and experiments have been conducted on more adult human subjects. The previous finding that coconut oil possesses a decalcifying effect has been verified. A set of typical data is appended :

Experimental subject	Name of oil or fat	Dietary calcium mg.	Faecal calcium mg.	Urinary calcium mg.	Balance mg.
S.C.D.	Fat free	485	351.3	165.8	—32.1
	Mustard	487.3	330.6	62.5	94.2
	Coconut	482.7	508.2	88.4	—113.9
	Sesame	492.5	328.6	127.9	36
	Groundnut	503	312	79	112
	Buffalo-butter fat-	497	261.3	53.4	182.3

(3) *Comparative rate of growth of young rats on different fats and oils.* Experiments have been conducted to determine the comparative rate of growth of young rats, consuming a diet in which all the constituents were the same except the oil which was different in different sets. The oils and fats studied were mustard, coconut, sesame, ground-nut and buffalo-butter fat. The composition of the diet was as follows :—Sugar—9 parts ; salt mixture—4 parts ; calcium carbonate—1 part ; agar—1 part ; fat free egg powder—10 parts ; oil—6 parts and starch—69 parts. It has already been reported that administration of fats to young rats at 6, 9 and 12% level showed that the maximum rate of growth was induced at 6% level of fat intake. In addition to the above diet each rat was supplied with yeast extract and adexolin. The experiment was continued for 8 weeks. Amongst the vegetable oils studied ground-nut oil effects greatest increase in wt. per gm. of the oil fed. The following are the typical results :—

Dietary fat	Increase in wt. per gm. of the oil consumed.
Mustard oil ..	.. 1.55
Coconut oil ..	.. 1.48
Sesame oil ..	.. 1.3
Ground-nut oil ..	.. 1.76
Buffalo butter fat ..	.. 1.97

(4) *Stability of vitamin A in different oils.* Stability of vitamin A in different oils has been studied. For this purpose a sample of vitamin A (in the form of shark-liver oil obtained from Coonoor) was diluted with mustard, coconut, sesame and ground-nut oils. It has been found that the vitamin is

completely stable in the refrigerator for about five months, but when kept at room temperature the vitamin potency of the oils deteriorates. During the first three months the deterioration, except in the case of sesame oil, is not very great. There is a considerable deterioration in the subsequent two months and after this period the vitamin A activity of the sesame oil sample is completely lost.

(5) *Effect of low fat diet on the human subject.* Experiment was conducted on a healthy, normal, adult human subject to determine the effects of a diet extremely low in fat. The diet was the ordinary vegetarian (rice, pulse and vegetables) diet without fat, with liberal supplements of lemon juice and sugar and contained about 2 gms. of fat derived mainly from rice and vegetables as compared with 40–50 gms. in a normal diet. Water was allowed ad libitum. The experiment was conducted for a period of 2 weeks during which period the subject was carefully observed. He did not feel any inconvenience but the food consumption was slightly lower than normal and the weight gradually diminished (by about 10 lbs.). Estimation of blood lipid content and the iodine absorption number of the total lipids before and at the end of the experiment revealed that blood lipid content became slightly lower and the iodine value also fell slightly.

Further experiments are in progress.

Mineral metabolism.

The estimation of the availability of iron of different food-stuffs is being continued by direct experiments on human subjects as well as by determining the efficiency of foodstuffs in regenerating haemoglobin in young rats made anaemic by rearing them only on milk. The total intake of iron in a typical 'Rice fish' diet was found to be 20.45 mgs. and urine and faeces excreted 2.05 mgs. and 12.2 mgs. of iron respectively. According to Hill's α-α^1-dipyridyl method only 3.45 mgs. of available iron was found in the above diet. Urine gave absolutely no colour indicating the total absence of 'available' iron while the method could not be applied to the estimation of iron in faeces. It appears, Hill's method does not estimate the whole of available iron.

The mutual antagonistic action of different minerals in metabolism reported earlier is being further studied in more human subjects. The effect of different vitamins on the metabolism of different minerals in man is also being further investigated.

Various factors of vitamin-B complex.

The thiochrome method for the estimation of vitamin B_1 has been standardised and set up.

The "Chastek paralysis" factor present in fish is under investigation. Some common varieties of fish e.g. Ruhee (Labeo Rohita), Katla (Katla Katla), Hilsa are being investigated. The question whether intestinal micro-organisms produce appreciable amounts of vitamin B_1 in human beings is also being studied.

The Coonoor method for the estimation of nicotinic acid with some modifications has been set up and work on nicotinic acid metabolism is in progress.

Soya-bean.

Biological value of soyabean protein :—

Experiments on the biological value of proteins of the third sample of soyabean received from Lahore at the end of 1943, and of a Bengal sample received

from the Dacca Government Farm were carried out by the balance-sheet method on adult rats at 10 p.c. level of protein intake. The values are tabulated below. The corresponding values for other two varieties (Kashmir and Lyallpur) as already reported last year are also given below for the sake of comparison.

Name of sample.	Protein content.	Biological value.	Digestibility.
Kashmir sample of soyabean ..	41.9	51	91
Bengal soyabean ..	40.85	50	90
Manchurian soyabean ..	41.6	44.1	89
Lyallpur soyabean ..	41.2	48.1	90.5

Effect of heat treatment on the biological value of pulse and soyabean protein :—

The pulses Bengal gram, green gram and three varieties of soyabean were autoclaved for 2 hours at 125°C. The biological values and digestibility of the proteins were found by the balance-sheet method on a series of adult rats. The results are as follows :—

Name of sample.	B.V. after autoclaving.	B.V. before autoclaving.	Digestibility after autoclaving.	Digestibility before autoclaving.
Manchurian soyabean	63.6	44.1	91.5	89
Kashmir soyabean	64	51	94	91
Lyallpur soyabean	63.8	48.1	94	90.5
Bengal gram	59.3	63	92	94
Green gram	38.6	49.8	83.2	91

Effect of supplementing poor rice diet :—

(a) Experiments on the supplementing effect of various pulses and different varieties of soyabean on poor rice diet were carried out on a series of young rats. As supplement to the poor rice diet, 3 parts by weight of pulse or soyabean were added to 22.5 parts of rice diet.

The mean weekly increase in body weight of rats are as follows :—

Rice diet alone	.. ..	.. 2.0 gms.
,, ,, and Kashmir soyabean	.. ..	.. 6.25 gms.
,, ,, and Lyallpur soyabean	.. ..	.. 4.0 gms.
,, ,, and Manchurian soyabean	.. ..	.. 3.2 gms.
,, ,, and Red gram	.. ..	.. 3.4 gms.
,, ,, and Bengal gram	.. ..	.. 3.5 gms.

(b) Another series of experiments on the supplementing effect of different varieties of soyabean and pulses on rice diet to which calcium-lactate, vitamin-B complex (as yeast extract) and shark-liver oil were added according to the plan agreed upon have been completed. As supplement to a poor rice diet, from every 22.5 parts of rice diet mixture 3 parts by weight were replaced by 3 parts of pulse or soyabean.

The mean weekly increase in the body weight of the rats is reported below :

Rice diet	alone	.. 6.1 gms.
„ „	and Kashmir soyabean	.. 8.7 gms.
„ „	and Lyallpur soyabean	.. 7.6 gms.
„ „	and Manchurian soyabean	.. 5.5 gms.
„ „	and Red gram	.. 7.2 gms.
„ „	and Bengal gram	.. 7.5 gms.

(c) A third series of experiments on the supplementing effect of different varieties of soyabean and pulses after autoclaving, on poor rice diet have been performed. As supplement, from every 22.5 parts of rice diet mixture, 3 parts by weight were replaced by 3 parts of autoclaved soyabean or pulse powder. Calcium-lactate, yeast extract and shark-liver oil were added as before.

The mean weekly increase in the body weight of rats is reported below :

Rice diet	alone	.. 5.9 gms.
„ „	and Kashmir soyabean	.. 9.1 gms.
„ „	and Lyallpur soyabean	.. 8.0 gms.
„ „	and Manchurian soyabean	.. 6.8 gms.
„ „	and Red gram	.. 6.4 gms.
„ „	and Bengal gram	.. 6.6 gms.

Effect of vitamin C on the biological value of pulse proteins by metabolism experiment on human subject :—

Experiments on a human subject (A.K.M.) have been conducted to determine the biological value of pulse and soyabean proteins and also the effect of administration of vitamin C on the biological value.

The results are reported below :

Name of sample.	B.V. without vit. C.	B.V. with vit. C.	Digestibility without vit. C.	Digestibility with vit. C.
Bengal gram	64	66	94	95.6
Red gram	60	63	90	92
Kashmir soyabean	59	61	92	95
Lyallpur soyabean	56.8	59	91	93
Manchurian soyabean	42	42	88	88.5

15. Nutrition research unit under Professor S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

The nutrition research unit started functioning from the 1st of April 1944. The enquiries on (1) Fat Metabolism and (2) Soya bean were absorbed in the unit which reached its full strength in personnel on the 1st of August 1944. The following is an account of the researches in progress.

1. Fat metabolism.

(a) *Growth tests :* A new set of experiments on albino rats in which the protein and salt intake of animals in all the groups was equalized, confirmed last

year's findings that rats grow well on a diet containing 5 per cent butter fat. The control diet contained only 0.85 per cent non-butter fat. Both the diets, however, were otherwise adequate with regard to the protective food factors. Increase in the level of fat intake beyond 5 per cent did not bring about any further improvement in the growth rate. The results are given in table I.

Table I.

THE EFFECT OF DIFFERENT LEVELS OF BUTTER FAT INTAKE ON THE GROWTH OF RATS.

Diet	Gain in wt. per week per rat	Average intake per day per rat		
		Food	Protein	Calories
	g.	g.	g.	
0.85 p.c. fat	10.27	8.2	1.67	32.97
5 p.c. „	11.70	7.9	1.73	33.55
12 p.c. „	11.0	6.8	1.75	29.83
19 p.c. „	10.94	6.5	1.70	32.24
25 p.c. „	10.6	6.1	1.70	32.02

An experiment to compare the effects of butter, hydrogenated ground nut oil and refined ground nut oil on the growth of rats is in progress.

(b) *Metabolism experiments :* The metabolic experiments on rats receiving butter fat at 5,12,19 per cent levels have shown that even at high levels of fat intake, the latter is almost completely absorbed from the intestine. The total nitrogen excretion in urine was found to bear approximately the same ratio to the nitrogen intake at all levels of butter fat feeding. The value for ammonia nitrogen fraction of the total urinary nitrogen, however, showed an increase with increased fat intake.

Table II.

THE RESULTS OF METABOLIC EXPERIMENTS AT DIFFERENT LEVELS OF BUTTER FAT INTAKE.

Diet.	No. of animals	Daily food intake g.	Daily fat intake mg.	Total Lipoids in faeces mg.	Daily nitrogen intake mg.	Total N in urine mg.	Ammonia N in urine mg.	
0.85 p.c. fat	5	9.65	77	16.6	320	130	5.52	non-butter fat
5 p.c. „	6	10.75	538	28.7	342	157	6.00	butter fat
12 p.c. „	6	7.32	879	46.7	227	113	7.59	„
19 p.c. „	6	6.37	1210	53.1	196	95	9.43	„
25 p.c. „	4	7.37	1841	59.4	227	131	9.69	„

(c) *Reproductive function :* The rats receiving butter fat at different levels were mated on reaching maturity and it was observed that the animals on 5,12,19 and 25 per cent butter fat gave birth to litters about 21 days after mating, whereas the animals on fat poor diet did not bring forth any litters with the exception of one animal which died during the process of giving birth to young ones in her first litter. The litters from other groups, however, did not survive beyond 2 to 5 days. In trying to investigate the cause some additions to the diet were made. 0.75 gm. fat free dried yeast per rat per day was included in the diet in place of the watery extract of a similar quantity. This change increased the survival rate in many cases. All the young rats, however, were underweight as compared with the normals of the same age. Later on vitamin E was added to the diet in addition to 0·75 gm. dry yeast but with no better results. In an earlier experiment the histological examination of ovaries, tubes, and uteri of female rats and testes of male rats who had shown reproductive failure on fat poor diet had not revealed any evidence of vitamin E deficiency, nor was there any evidence of fat deficiency disease in any of the animals.

The frequency of litters in rats on 5 per cent butter fat was greater as compared with rats on higher fat levels.

(d) *Absorption from the intestine :* The rate of absorption of fats in human beings is being studied by Frazer's (J. Physiology 1939, **95**, 21 P) technique of chylomicron count. The fats studied till now have been butter, ghee, hydrogenated ground-nut oil and refined ground-nut oil. The results give a definite indication that the refined oil and its hydrogenated product are absorbed from the intestine at a much slower rate than are butter and ghee.

2. Soyabean.

In accordance with the recommendations of the Soyabean Sub-Committee new varieties of soyabean were compared for the biological value of the proteins with that of common pulse proteins. The Vitamin A, B_1, B_2 and nicotinic acid content of soyabeans and Bengal gram has also been investigated. In the following table is included some of the information thus gained.

Table III.

Biological value by metabolism experiment and growth test

	Metabolism tests.			Total protein content.	Available protein per cent	Biological value by growth test per cent.
	Nitrogen Intake per cent	Biol. value.	Digest. coeff.			
Soyabean Manchurian	1.61	55.9	76.2	41.6	17.7	1.22
Soyabean Lyallpur	1.76	63.4	84	36.2	19.3	1.63
Soyabean Kashmir	1.71	64.9	92.2	44.9	26.9	1.24
Bengal gram	1.59	69.5	84.6	24.7	14.6	1.68
Green gram	1.52	48.5	86.2	27.5	11.5	..
Tur Dal	1.55	54.7	76.1	22.3	9.3	..

It had been felt that the knowledge about the essential amino acid content of soyabean was not at a satisfactory stage. The work on this aspect, therefore,

has been undertaken and it is expected that once the methods have been standardized the investigation of amino-acid content could be extended to other foodstuffs as well.

3. Analysis of foodstuffs.

The analysis of certain foodstuffs, not commonly known outside the province has been undertaken. The investigation is intended to give information on the proximate principles of the foodstuffs, their vitamin content and the biological value of the proteins of some of them which are found to be useful sources of proteins.

4. Researches on vitamin D.

These investigations are in continuation of the work carried out earlier in these laboratories on calcium and phosphorus metabolism. They are designed to throw light on the mode of action of vitamin D. The problem is being attacked from two angles (a) a study of induced rickets in dogs and rats and (b) studies *in vitro* of metabolism of the epiphyseal cartilage by the micro-respiration technique.

16. Enquiry into methods of treating sick starving destitutes in Calcutta based on the All-India Institute of Hygiene and Public Health, Calcutta.

A. School of Tropical Medicine

(i) *Incidence of malaria*—8110 cases among the sick destitutes were examined for malarial parasite, of which 35.2% was positive. Of late the incidence is increasing rapidly.

(ii) *Incidence of bowel diseases*—Out of 682 cases examined 35.6% had bacterial infection.

(iii) *Haematological investigations.*

(a) Complete cytological examination of blood was done on 103 cases. Of these 80 cases were indoor patients in the Carmichael Hospital for Tropical Diseases and the rest were from different hospitals. Repeated examinations (323 times) of blood were done on the cases in the Carmichael Hospital. Some degree of anaemia was present in all the cases. It was severe in 20%, moderate in 61% and slight in 19% of cases. The nature of anaemia was macrocytic in 34%, normocytic in 46% and microcytic in 20% of cases.

Total and differential counts were done in a large number of cases and were found generally to be of normal order.

(b) *Van den Bergh test* was done in 98 cases. The direct test was positive in 3 cases only and the indirect test in 38 cases. In the latter series only in 5 cases was the value above the normal range.

(c) *Prothrombin time* was estimated in 35 cases and was found to be definitely high in 2 cases only.

(d) *Fragility test of R. B. Cs* was done in 32 cases of malaria during febrile paroxysms. The findings were within normal range.

(e) *Specific gravity of blood* was determined in 13 emaciated cases, in all of which it varied from 1040 to 1050.

(f) *Examination of gastric juice*—Fractional gastric juice analysis was done in 25 cases. The results are as follows :

1.	Hyperchlorhydria	4
2.	Hypochlorhydria	10
3.	Achlorhydria (without histamin)	3
4.	Absolute achlorhydria (after histamin)	1
5.	Within normal range	7

(g) *Examination of marrow material by sternal biopsy.* The condition of the bone-marrow was investigated by sternal biopsy in 34 cases. The marrow appeared normal in 9 cases, hypoplastic in 7, megaloblastic in 1 and in the remaining 17 cases it was plastic and normoblastic in reaction.

(iv) *Biochemical investigations.*

BLOOD.

Sugar—Blood sugar (fasting) was estimated in 90 cases. In 25% of the cases it was very low, in 48% of the cases the result was low, and in 27% of the cases the blood sugar was within normal limits.

Total protein—Total content of protein was estimated in 140 cases. In 8% of the cases the protein content was normal. In 92% of the cases the protein value was low. Albumen/globulin ratio—in 50% of the cases the ratio was 1:2 and over. In 25% of the cases it was 2:1 and in 25% of the cases 1:1.

Non-protein nitrogen—Non-protein nitrogen was estimated in 62 cases. In 23% of the cases the result was within normal limits. Results below normal were obtained in 21% of the cases and 56% of the cases showed result above normal.

Urea—Variable results were obtained generally on the higher side.

Plasma chlorides—Chlorides was estimated in 34 cases. In 40% of the cases the result was below normal. In the rest of the cases the result was within normal limits.

Cholesetrol & Lecithin P—Cholesterol and lecithin P content of the blood was done in selected number of cases. The result obtained was low as regards cholesterol but high as regards Lecithin P.

C. S. fluid—Some samples of C. S. fluid were examined for sugar, chloride and protein.

Urine—Complete examination of the urine was done in 47 cases. In 25 cases the urine chloride was estimated and was found to be low. Albumin was present in traces in almost all cases.

Carmichael Hospital for Tropical Diseases.

(v)	Total number of admissions	72
	Total number of deaths ..	6
	Total number of discharged (cured or improved) ..	48

Malaria—Various strengths of the dose of quinine were tried on these cases. All were ultimately cured excepting one who died of hypertensive cardiac failure. Recently a new drug (M 3349) of I.C.I. is being tried.

Kala-azar—3 cases were diagnosed as kala-azar. Two responded well with treatment and the third one is still under treatment.

Bronchitis and broncho-pneumonia.—7 patients developed this complication and 5 of them were treated with specific drug. The other 2 patients died.

Dysentery—Routine examination of stools in all the cases showed *E. histolytica* in 3 cases, vegetative forms in 2 and cysts in the third. Three cases showed symptoms of bacillary dysentery which on examination of the stools showed cellular exudates but no specific organism could be isolated. All these cases responded well to treatment with sulphaguanidine.

Tuberculosis—Tuberculosis was detected in 4 cases, one of whom died.

Oedema—There were 26 cases of oedema including 3 cases of nephritis. These cases were generally more resistant to treatment than the cases treated last year. Definite improvement was seen in all cases excepting the 3 cases of nephritis. Rest and hospital diet alone produced improvement in 6 cases; while most of the remaining cases responded to preliminary draining of the tissue fluid by mercurial diuretics e.g. neptal, followed by a high protein diet. Almost all the cases had low serum protein with altered albumen and globulin ratio.

Enlargement of liver—Slight or moderate enlargement of liver was noted in a number of cases. In some enlargement was progressive and there was marked dilatation of veins over the chest and abdomen. Prothrombin time, excepting in one was within normal range. *E. histolytica* infection in these cases was excluded by repeated stool examination including culture for *E. histolytica.*

Anaemia—Some degree of anaemia was found in all the cases. In some cases improvement was slow inspite of the usual haematinics. Blood transfusion was given in 4 cases with good results. Oral preparation or proteolysed liver extract prepared by the "Enquiry into the fraction of liver most suitable for the treatment of tropical macrocytic anaemia" at the School of Tropical Medicine, was tried in 10 cases of macrocytic anaemia. Marked improvement was noticed in 2 cases and moderate improvement in all the rest except one.

B. Campbell Hospital.

Total number of cases investigated were 90. Of these 62 (approx. 68%) had oedema, and 57 (approx. 63%) had bowel complaints, and as many as 29 (approx. 30%) had both.

Of the 62 cases grouped under the general heading oedema only 12 could be accounted for, by a definite kidney, heart or liver condition. Of the remaining 50 cases, 41 had in addition a severe degree of anaemia (Haemoglobin value as low as below 30% Helige being present in many). 15 of the latter group of 41 cases had either chronic malaria or ankylostoma duodenale infection to account for their anaemic state. The remaining 26 cases make very interesting study, the point for investigation being whether the severe degree of anaemia was responsible for the oedema or both anaemia and oedema had their origin in prolonged nutritional deficiency. The latter seems to be more probable.

Of the 57 cases admitted with bowel complaints, bacillary dysentery, amoebic dysentery and tuberculous enteritis were responsible for 20 (approx. 35%), 10 (approx. 18%) and 3 (approx. 5%) cases respectively. Cysts of giardia intestinalis were found on routine examination of stool in quite a number of cases. But in 19 (approx. 33%) cases no cause could be ascertained. Almost all of these 19 cases having evidences of marked malnutrition, assumption is that they were cases of nutritional diarrhoea.

C. All-India Institute of Hygiene and Public Health.

Postmortem materials were collected from the Medical College mortuary and also from the Campbell Hospital mortuary. The tissues were fixed in Zenker's fixative, dehydrated and embedded in paraffin under vacuum to expedite the work. Sections were cut by process as modified by De. Postmortem study of 25 cases has so far been made and is recorded briefly under the following two headings.

1. *Gross abnormalities.*—The general appearance is that of a markedly emaciated and moderately anaemic person with dry lustreless skin. Some degree of oedema of the subcutaneous tissue particularly around the ankles, on the dorsum of the feet is common. Increased pigmentation of skin with collection of dirt, particularly at the flexural areas is another common external finding. The subcutaneous fatty tissue and the muscles are considerably thinned out. In almost every case, there is found an appreciable collection of clear straw coloured fluid in the peritoneal cavity and in the pericardial sac, more particularly, in the former. The mucosa of the small intestine is markedly congested, in its lower 2 to 6 feet. In some cases, it shows patchy submucous haemorrhages. The plicae circularis of the intestinal mucosa are obliterated at places. The mucous membrane of the pylorus and the duodenum, particularly of the 1st and 2nd parts of the latter is markedly congested and at times also present submucous haemorrhages. Liver is shrunken in size with the capsule somewhat wrinkled. Spleen is also shrunken in size with the capsule presenting a wrinkled appearance. In cases where malaria is present in addition, enlargement, congestion and slaty pigmentation of the liver and the spleen are seen. The kidneys are congested but present no other abnormality. The supra renals do not show any gross abnormality. The pleural cavities are commonly obliterated completely or partially by the development of adhesions between the visceral and parietal pleurae. Such adhesions are usually easily breakable. The lungs show evidence of congestion and oedema. In addition in some cases evidence of tuberculosis are met with. The latter is chiefly of fibrocascous type. Generalised dissemination of tuberculosis or miliary affection of the lungs are not seen. The heart size is smaller than normal but relatively the right heart is dilated and engorged. The brain does not present any obvious gross abnormality.

2. *Histological findings.*—Cloudy swelling of the liver and the kidneys was a common finding. Congestion of marked degree was met with in sections of the lungs and the kidneys. In addition in some of the cases, the lung alveoli present evidence of exudate, chiefly composed of mononuclear cells. The intestinal mucosa shows denudation of epithelium particularly over the villi. The villi themselves are implicated with mononuclear cells, to an abnormal extent. It appears that the cholesterol contents of the supra renals diminish, as the usual vacuated appearance of the cells of the cortex is not conspicuous. Liver and spleen show evidence of congestion and in cases where malaria was co-existent, deposits of haemozine pigments are also found in the cells of the reticuloendothelial system.

Department of Applied Chemistry, University of Calcutta.

Blood samples were examined for different items, specially to get a detailed account of the nitrogen picture of the blood. The significant abnormal findings are :—

(i) High N.P.N. with abnormally low urea N and with an enormous increase in free ammonia N Value.

(ii) Abnormally low Hb and chloride value.

(iii) Low serum albumen with proportionate decrease of globulin.

17. Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.

Under this enquiry the different carotinoid pigments in plant foods are being isolated and individually estimated. Generally the number of pigments vary from 5 to 10, so that if only a total estimate of the pigments is made as has been done by most workers in this field, it gives an erroneous idea of vitamin A activity, since only a few of them are active. The work involves the difficult technique of repeated chromatographic separation, identification by means of absorption spectra, and estimation of the pigments by means of Nuttings—Hilger Spectrophotometer.

15 samples of maize of different varieties collected from different areas of the Punjab have been examined and found to contain 7—8 different pigments in varying proportions. The following is the summary of the typical results :—

Cryptoxanthin	.. 0.49 — 7.74	ug per g.
Neo-cryptoxanthin	.. 0:31 — 1.37	„
β— Carotene	.. 0.17 — 3.85	„
∝— Carotene	.. 0.17 — 0.68	„
γ— Carotene	..	
k— Carotene	.. 0.18 — 0.50	„
Zeaxanthin	.. 1.24 — 28.70	„
Unidentified pigments	.. 0.15 — 1.87	„

It will be seen from the results that the first six pigments which are presumably biologically active represent only 31.6 to 47.3% of the total pigments present. Similar studies on other plant foods are in progress. It is also intended to study the concentration of different pigments in different tissues at different periods of growth, and the changes they undergo on storing. This work is likely to give information on the fundamental question of the course of carotenoid metabolism in plants.

18. Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

Portal cirrhosis of the liver is a common disease in certain parts of India and its aetiology is obscure. Its incidence in the Northern Circars of the Madras Presidency is fairly high and preliminary investigations carried out during the years 1931 to 1935 showed that "alcohol appears to have a very insignificant role" and that "dietetic deficiencies appear to be important factors in the causation of cirrhosis of the liver in the Circars". The diets of patients suffering

from the disease were grossly deficient in protein, fat and vitamins, especially vitamins A, B_2 complex and C. Syphilis, chronic bacillary dysentery and splenic anaemia appeared to be important contributing factors in the causation of the disease.

During recent years, experimental evidence has accumulated to suggest that cirrhosis of the liver may be a deficiency disease. It has been shown in animals that deficiency of protein and vitamins of the B_2 group in the experimental diets produced necrosis of the hepatic parenchyma and occasional periportal fibrosis.

The enquiry which was commenced in June, 1944, was undertaken to study the part played by dietary factors in the causation of cirrhosis of the liver.

Experimental hepatic cirrhosis.

Experiments to study the effects on liver of (1) continued deprivation of all the factors present in vitamin B_2 complex, (2) varying levels of protein intake, and (3) low protein intake combined with a deficiency of the vitamin B_2 complex, are in progress and will be continued next year. In the first experiment, young healthy albino rats are fed on purified synthetic diets deficient in vitamins of the B_2 complex but complete in other respects. To a control group of animals, whole dried brewer's yeast, to supply all the B group of vitamins, was added to the diet. In the second experiment, the level of protein (purified casein) has been reduced from 18% level in the first experiment to 5% and 10% respectively in two different groups of animals. With the exception of this difference in the protein level, the composition of the diets is similar to that given to the control group in the first experiment. The third experiment is similar to the second, except that whole dried brewer's yeast which was included in the diets of the latter was not given to the rats in this group. The diets were, however, supplemented with thiamin, to meet the vitamin B_1 requirements. It is proposed to study the histological changes in the liver in the above groups by the application of different staining methods.

Preparation of methionine.

Evidence is now accumulating to suggest that the amino acid methionine plays an important part in the prevention of experimental hepatic necrosis. As pure methionine is not available at present in India, preliminary work has been undertaken to isolate the amino acid from casein hydrolysate. This work is in progress.

Preparation of yeast.

Owing to the difficulty of obtaining good brewer's yeast for use in experimental and clinical investigations, the possibilities of laboratory production of yeast are being explored and the work will be continued during the coming year. Three strains of *Torula utilis* were collected and the different factors which govern their growth in molasses solution are being carefully investigated.

19. Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

1. Nature of egg-iron

Previously it has been shown that about 30-40% of the tissue iron (animal tissue) is present in complex combination with nucleoprotein and copper and this iron can be quantitatively estimated by Hill's dypyridyl method. This complex iron has been found to be normal constituent of all red-blooded animals hitherto investigated. A comparative study on the egg-yolk iron and tissue iron reveals some striking differences in their chemical behaviour. These differences are enumerated below and may be due to the differences in their chemical nature.

(a) The amount of iron liberated from the same sample of duck's egg-yolk by the action of sodium hydrosulphite, thiol-acetic acid or sodium pyrophosphate is the same and it agrees fairly with the quantity of iron obtained after ashing. The trichloroacetic acid control gives negative test for iron. But in the case of fish tissue, the iron value obtained by the reduction with sodium hydrosulphite at pH 5.5 is less than that obtained by thiolacetic acid, but if the sodium hydrosulphite is added after treatment of the tissue with 8% acetic acid by Hill's method as modified by Saha and Guha (1940, *Ind. Jour. Med. Res.* 27, 877) then the value becomes nearly equal to that obtained with thiolacetic acid. Treatment with sodium pyrophosphate gives only 30 to 40% of the iron. Trichloroacetic acid extract of the control gives the test for iron, the amount being very small. Moreover, the amount of iron obtained after ashing is always greater than that obtained by any of the above methods.

(b) Iron added to egg-yolk cannot be recovered by enzymic hydrolysis, whereas in fish and other animal tissues, added iron can be quantitatively recovered from the hydrolysate.

(c) Enzymic hydrolysis and increase in acidity do not increase the iron value of egg as in the case of fish and other animal tissues.

2. Haemopoietic effect of nucleic acid on rats kept on milk diet.

The haemopoietic action of a considerable number of chemical substances in relation to anaemia has been investigated by various workers. The authors have been particularly concerned with nucleic acid as a haemopoietic factor, as they obtained a preparation from fish tissue, which appeared to be iron-nucleotide and it was of interest to study haemopoietic value of this substance as well as of nucleic acid in nutrition. Nucleic acid is an essential constituent of all living cells and is synthesised inside the system and, so far as the present knowledge goes, exogenous supply of this material seems to be unnecessary. Still, it has been observed that if this substance is injected into normal or anaemic vertebrates marked haemopoiesis results.

From the results it is clear that both the haemoglobin and erythrocyte formations in the rat are greatly enhanced by supplementing iron and nucleic acid to a basal milk diet. The total iron content of the body in this group of animals seems to be slightly higher than what has been reported by Shythe and Miller (1929) J. Nutrit., **1**, 209 for animals of the same age and body-weight. They stated that the amount of iron in the rat body which is highest at birth,

viz. 0.0055% of the body-weight, drops to 0.0026% after 20 days and then rises to 0.0043% after 100 days and for adult rats on a commercial calf-meat diet the iron content averages about 0.0051%. The iron values obtained with the calf-meat diet agrees fairly with our findings, probably due to the fact that calf-meat contains a high percentage of nucleic acid which makes the iron more available than the ordinary normal diet.

On administering nucleic acid alone, stimulation of haemopoiesis results up to the second week, after which both the blood haemoglobin and erythrocyte count fall abruptly to a low value and then upto the 5th week the haemoglobin content falls more slowly but the erythrocyte count maintains almost a steady value. In this group of animals the total iron content of the rats falls below the normal. These results indicate that so long as the stored iron of the body can supply the need, its mobilisation for the purpose of haemopoiesis is stimulated by nucleic acid, and then due to the shortage of stored iron both the haemoglobin and the R.B.C. contents fall abruptly to a low value. It has also been found that the blood coagulation time increases considerably after the expiry of the second week. It would thus appear that, so long as the body-store is sufficient, nucleic acid supplement would stimulate haemopoiesis and erythropoiesis, but when the store is very much reduced the effect of nucleic acid may be deleterious. It would follow that in those anaemias where tissue iron is greatly depleted, nucleic acid, unsupplemented by iron, may have indeed an injurious effect. On the other hand, administration of nucleic acid plus iron appears to supplement milk effectively for haemopoiesis and erythropoiesis. It is likely that these supplements would have similar action in both normal and anaemic cases.

It is interesting to note that the addition of iron to milk diet has no effect on the haemoglobin or erythrocyte content of the blood but increases the total iron of the body to a great extent. These findings are in close agreement with those of Elvehjem and Sherman (1932, *J. Biol. Chem.* 98, 309) which indicate that addition of pure iron to the milk diet of anaemic rats, which had been well depleted in their reserve of iron had no effect on the haemoglobin content of the blood, but increase the total iron content of the liver and spleen to a large extent. It would appear, therefore, that milk cannot be supplemented effectively for haemopoiesis by iron alone.

When the rats were kept on milk as the sole source of diet, the haemoglobin and the R.B.C. content of the blood maintained almost a steady level upto the 3rd week of the experiment, probably at the expense of the stored body iron and when the reserve iron reached limiting value, the above values began to decrease slowly. The total body iron fell below the normal level. No sign of definite anaemia (a value of haemoglobin below 70 per cent) developed, however, even in the 5th week.

As all these experiments were carried out on the animals which were on milk diet for 5 weeks and had not developed anaemia, it is clear that the haemopoietic and erythropoietic action of nucleic acid plus iron is effective under normal physiological condition. It is probable that it is also effective at least in some types of anaemic conditions.

Addition of nucleic acid to milk diet does not, however, promote growth which is clear from the fact that the mean increment of body-weight is the same in all the cases.

20. Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras.

Animal experiments.

Experiments with rats have been conducted (1) to demonstrate for educational and propaganda purposes the inadequacy of poor rice diets, (2) to test the effects of supplements to rice diets, (3) to compare South Indian cereals, and (4) to explore modifications of rice diets that do not involve increase in cost. Small scale experiments were set up with series of pairs of litter mates of young weaned rats and daily records were kept of food consumption.

(1) *Poor rice diets.* The basal "poor rice diet" consisted of parboiled milled rice (*cooked*) 89.7 per cent, Bengal gram (cooked) 3.0 per cent, brinjal 4.3 per cent, mutton 0.26 per cent, coconut 0.21 per cent, green plantain 2.1 per cent, gingelly oil 0.43 per cent. This diet was given *ad lib.* On this diet growth of rats for the 8-week period from 4 to 12 weeks of age was very slight, averaging 1.7 gms. per week. (The average gain for stock animals in this laboratory for the same period is 16.8 gms. per week.) The general condition of the animals was also very poor as indicated by malformation of the hind legs, loose rough hair with extensive bald patches, and susceptibility to respiratory infection and death from pneumonia. Of the only 11 female rats on the diet that survived as long as 20 weeks none had litters.

(2) *Effect of supplements.* The following table shows the effects of supplements on growth for the same age period, 4 to 12 weeks.

Table I.

Effects of supplements to poor rice diets (cooked) on growth in rats.

Supplement.	Amount given gms. per rat per day.	No. of rats.	Average weekly increase, gms.
No addition ..	..	59	1.7
"Rasam"[1] (concentrated) ..	10.0	4	2.2
"Rasam" (dilute) ..	10.0	9	1.7
Cow's milk ..	*ad. lib.*	4	14.0
" " ..	12.5	15	10.5
Curds ..	12.5	5	3.6
Milk (½ diluted i.e. 6.25 gms. milk)	12.5	7	4.9
Curds (½ " " " " curds)	12.5	3	3.4
Calcium lactate ..	.115	8	6.1
Casein, technical ..	.4	4	4.1
Butter ..	.5	5	.9(2)
Ghee ..	.5	3	1.5(2)
Gingelly Oil ..	.5	2	1.4
Groundnut oil ..	.5	3	1.1
Coconut oil ..	.5	3	1.7
Shark liver oil ..	3 drops	5	1.6
Egg yolk ..	1.5	6	5.6
Amaranth ..	5.0	11	4.3
Drumstick leaves ..	5.0	3	3.7

Supplement.	Amount given gms. per rat per day.	No. of rats.	Average weekly increase, gms.
"Agathi Keerai" ..	5.0	3	3.0
Dhal arhar (cooked) ..	4.0	4	2.3
Ragi (Eleusine coracana) (60% rice+30% ragi) ..		8	7.9

Footnote: (1) "Rasam" is "pepper water" made from tamarind, onion and minute quantities of chillies, garlic, mustard, pepper, cumin and gingelly oil.

(2) The difference in effects of butter and ghee is at least partly explained by the appetite factor. Rats with butter experiments ate less of the basal diet than with any other supplement.

These results of small experiments indicated : (1) that calcium and natural foods rich in calcium—milk, ragi, amaranth and other leafy vegetables—were valuable supplements to rice diets. Curds, also a source of calcium, showed less good supplementary effects than unsoured milk but this was partially explained by the appetite factor ; (2) that rasam, one of the most common accompaniments of rice diets had no supplementary value except to prolong life ; (3) that the addition of *fat* to the 90 per cent rice diet, in the form of butter, ghee, shark liver oil or vegetable oil, not only induced no supplementary effect on growth but worsened the general condition of the animals.

(3) *Comparison of South Indian cereals.* The striking effect observed with ragi was compared with other cereals with results as follows :—

Table II.

COMPARISON OF SOUTH INDIAN CEREALS FOR GROWTH IN RATS.

Diet (all cereals cooked)	No. of rats.	Average weekly increase, gms.
Rice (90% rice)	59	1.7
Ragi (90% ragi)	4	4.4
Cholam (jwar) (90% cholam)	6	1.2
Cambu (bajra) (90% cambu)	2	2.8
Rice (60%) + ragi (30%)	8	7.9
Rice (60%) + cholam (30%)	2	3.0
Rice (60%) + cambu (30%)	2	2.4
Rice (60%) + wheat (30%)	2	3.8
Cholam (60%) + ragi (30%)	7	6.2

These results inidcated that cholam and cambu substituted for part or all of the rice had insignificant supplementary effects and little if any superiority over rice as a basal cereal. When ragi was used as the basal cereal alone the growth and condition were better than with any of the other cereals, but not so good as when used *in combination* with rice or with cholam.

(4) *An improved cheap modification of rice diet.* An "experimental school diet" of parboiled milled rice (cooked) 50 per cent, ragi (cooked) 20 per cent,

Bengal gram (cooked) 10 per cent, amaranth 10 per cent, sprouted green gram 5 per cent, brinjal 5 per cent, *costing no more than the poor rice diet* was found to induce strikingly better growth (7.5 gms. per week) and improved general condition. In striking contrast with the poor rice diet, 100 per cent of the female rats on this diet had litters. This indicates that marked (though still far from ideal) improvements can be made in poor institutional diets without increase in cost.

South Indian food preparations. Analyses of common food preparations in use in South India are much needed by dietitians. Analyses of moisture, protein, fat, total ash, calcium, phosphorus and calories have been completed for puri, vadai, uppuma and iddaly. The analyses were made from four samples of each purchased from at least two different hotels and from one sample prepared in the laboratory ("home-made" sample). Since hotel samples in some cases differed significantly from carefully controlled laboratory preparations and suggested adulteration the results are given separately.

Table III.

ANALYSES OF SOUTH INDIAN FOOD PREPARATIONS.

Preparation.	Moisture gm. %	Protein gm. %	Fat gm. %	Mineral matter gm. %	Calcium gm. %*	Phosphorus gm. %*	Calories 100 gm.
Puri (whole wheat flour and salt, fried in ghee).							
Mean of hotel samples ..	24.0	8.992	14.36	1.401	.0485	.1290	377
"home-made" sample ..	27.61	9.142	9.49	2.098	.0474	.0745	337
Vadai (black gram, green chillies, onions, ginger, curry leaves, coriander leaves, salt, fried in groundnut oil)							
Mean of hotel samples ..	38.36	12.74	14.13	2.726	.0648	.0410	302
"Home-made" samples ..	49.58	12.92	6.161	3.878	.0575	.0335	211
Uppuma ("Ravai"–Hindi name Sujji, black gram, cashewnuts, mustard, curry leaves, onions, green chillies, ginger, salt, gingelly oil)							
Mean of hotel samples ..	63.52	2.777	9.849	.9918	.0313	.0431	179
"Home-made" sample ..	57.85	4.178	12.75	1.554	.0719	.0756	252
Iddaly (parboiled milled rice, black gram, salt).							
Mean of hotel samples ..	69.75	4.177	(.0150)**	.7764	.0193	.0453	(93)**
"Home-made" sample ..	68.04	4.249		1.240	.0181	.027	

*Iron analyses were not completed because amyl alcohol was not available.
** Analyses incomplete.

LEPROSY.

21. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

During the year work was carried along the following lines :—(1) Clinical, (2) Immunological, (3) Therapeutic, (4) Epidemiological, and (5) Bacteriological. The following are the outlines of the work done.

Clinical.

The long term study of selected cases of leprosy has been continued. The cases are selected because of the presence of some atypical feature in the lesions. The purpose of the study was to correlate the clinical, bacteriological, histological and immunological findings with the prognosis of the disease in these cases. In the study are included cases with the following types of lesions (a) lesions of doubtful classification, (b) localised skin lesions of the lepromatous type, (c) flat patches of the neural type with ill-defined margins, and (d) tuberculoid patches of neural type with ill-defined margins and smooth and shiny surface. In addition, cases with the following types of lesions have also been included (e) tuberculoid lesions in phase of reaction, (f) lesions of all types among the children, and (g) lesions suggestive of leprosy but with no definite signs.

The grouping of the cases is done on the clinical findings, and it is proposed to follow them up over a long period, making repeated clinical, bacteriological, histological and immunological observations. There is bound to be some overlapping in the groups. The histological and immunological findings may later indicate transference of a case from one group to another, but for the purpose of the study the original grouping is being maintained.

Several cases have been under study for over a year or more, and others for a shorter period. It is yet too early to arrive at any definite conclusions. The following general observations may, however, be made.

(a) *Cases of doubtful classifications.* A large majority of the cases of leprosy can be classified as neural or lepromatous on clinical grounds and the prognosis of the disease in these cases can be stated with a fair degree of accuracy. There are, however, a small percentage of cases in which the clinical findings are not clear cut, the lesions being typical neither of the tuberculoid nor of the lepromatous type, but having some features of both the types. It is difficult or impossible to make a proper classification or prognosis in these cases. In some of these cases a histological examination of the lesions clears up the classification, but often atypical clinical findings are associated with atypical histology.

The lesions in these cases resemble the tuberculoid lesions in being localised, but are smooth and shiny with ill-defined margins and are strongly positive bacteriologically.

Of the cases under study in this group, lepromin test was positive in some, doubtful in a few and negative in others. Histologically the lesions in all the lepromin positive cases were tuberculoid although not quite typical, the atypical features being the presence of scanty giant cells and marked vacuolation of some epitheloid cells. In the lepromin doubtful and negative cases, histologically the lesions were atypical tuberculoid in some, lepromatous in others, and doubtful in a few others.

It appears that the lepromin positive cases in this group are really tuberculoid cases, the atypical clinical and histological findings being caused by the phase of reaction. Several of the lepromin negative cases in the group appear to be really lepromatous, but the status of some lepromin negative and doubtful cases in the group is not yet clear.

Our study has so far shown, that in some of the cases the atypical clinical and histological findings are temporary, and are often associated with phase of reaction. In some other cases, however, the atypical findings persist. The lepromin test appears to be of definite value in sorting out cases of doubtful classification.

(b) *Atypical lepromatous cases.* In this group are included the cases of lepromatous type in whom some or all the lesions were localised. (The lesions in the lepromatous cases are typically diffuse and generalised and not localised). All the lesions were strongly positive bacteriologically.

The lepromin test was positive in only one case, doubtful in a few, and negative in others. In the lepromin negative and doubtful cases, histologically the lesions were lepromatous with a tendency to tuberculoid arrangement of the granuloma cells. In the single lepromin positive case in this group, however, the lesion was histologically tuberculoid although not quite typical, as there were no definite giant cells and there was marked vacuolation of some cells. It appears that this lepromin positive case was really a tuberculoid case in phase of reaction, and not a lepromatous case with localised lesions.

(c) *Flat patches of the neural type with ill-defined margins.* The patches of the neural type whether thick (tuberculoid) or flat (simple) usually have a well defined out-line. In some cases, however, the margins are ill defined, and the patches are not so well circumscribed. Ill-defined patches, both flat and thick have been included in our study.

All the cases in the group of flat ill-defined patches have hypopigmented, ill-defined, anaesthetic lesions. The lesions in all the cases except one were bacteriologically negative. Lepromin test was positive in more than 50% cases and doubtful or negative in the others. Histologically the lesions were either simple with non-specific granuloma, or mild tuberculoid.

In some of the cases the lesions have faded slightly or to a great extent ; in some the disease has remained stationary, and in a small number of cases fresh lesions have appeared.

(d) *Tuberculoid patches of the neural type with ill-defined margins.* In this group are included the thick tuberculoid patches of the neural type with ill-defined margins. These patches may have a smooth shiny surface, and are usually bacteriologically positive, at times strongly. The lepromin test is positive in some and negative in others. The lesions in all the lepromin positive cases are histologically tuberculoid, though marked vacuolation of cells is seen. The lesions of the lepromin negative cases have been found histologically to be either lepromatous or doubtful, only rarely tuberculoid.

(e) *Tuberculoid cases in the stage of reaction.* In this group are included tuberculoid cases in which the history of the disease definitely indicated the presence of a reaction ; the disease had existed for some time but the signs of exacerbation were present only for the previous few months. From what has been said previously it would be clear that in addition to these cases, some of the cases included under cases of doubtful classification and amongst tuberculoid cases with ill-defined margins, were really cases of tuberculoid reaction.

Clinically the lesions in some cases were typically tuberculoid with well defined margins, in others the lesions were smooth with a shiny surface and an ill-defined margin. Histologically all the lesions were tuberculoid though not typical, the atypical features being the presence of a few or no giant cells, of marked vacuolation of some cells, and of little infiltrated nerves in the midst of foci of granuloma. All these cases have shown considerable subsidence of the lesions, and in some of them the lesions have become flat.

In several tuberculoid cases the onset of reaction appears to be associated with atypical clinical and histological findings. In some cases the lesions may become clinically so atypical that the classification of the cases as cases of the neural type becomes difficult or impossible. A number of such cases have been included in the group of doubtful classification. In such doubtful cases the atypical findings disappear with the subsidence of reaction ; however, there are other cases in the doubtful group in which the atypical findings persist.

(g) *Cases with suggestive but no definite signs of leprosy.* Under this group are included cases which have one or more hypopigmented, some times erythematous patches, looking like the patches of the leprosy but with no definite sensory changes and no leprosy bacilli. Most of these cases have been amongst children but some amongst adults also. In addition to the clinical, bacteriological, immunological and histological examinations, a histamine test is done in these cases. It appears that the histamine test is of great help in making a diagnosis in some of the cases. The two chief factors limiting the value of the test are the dark skin of some patients, and the erythema found in some patches.

Immunological.

(a) *Acid-soluble protein fractions of other acid-fast bacilli.* The study of the acid-soluble protein fractions of the various acid-fast bacilli was continued.

Our previous study had shown that the acid-soluble protein fraction of some acid-fast bacilli appeared to be similar antigenically to the similar fraction of Hansen's bacillus. Fractions from some other acid-fast bacilli have now been studied with similar results.

In most cases of leprosy intradermal injections of these fractions have given results similar to those given by lepromin, i.e., positive results in the neural cases, and negative results in the lepromatous cases. In some lepromatous cases, however, these fractions have produced a positive result. The hope of using these fractions for the lepromin test, in place of lepromin obtained from leprous nodules, has therefore not so far materialised. Further work in this connection is in progress.

(b) *Skin test with antigen from urine of cases of leprosy.* Some workers have reported that intradermal test with antigens prepared from urine of bacteriologically positive cases of leprosy provided a diagnostic skin test for leprosy, producing a positive result in all cases of leprosy, and a negative result in persons not suffering from leprosy. The matter has been studied, and the results obtained have not confirmed the above report.

Therapeutic.

(a) *Iodised hydnocarpus oil in the treatment of leprosy.* A study has been concluded regarding the suitability of iodine as a substitute for creosote for

addition to hydnocarpus oil for injections. The iodised oil has been found to be satisfactory, and even fairly large doses have not produced any bad effects in the cases treated with it. Simple addition of iodine to the oil is not sufficient, the iodine should go into chemical combination with the oil, and the finished product should not contain any free iodine. This can be achieved by carefully heating the mixture of oil and iodine at 140°C for half an hour, and then filtering the oil.

(b) *Sulphapyridine and rat leprosy.* A study has been concluded of the *in vivo* action of sulphapyridine (M.B. 693) on experimental rat leprosy. Under the conditions of the experiment, sulphapyridine has failed to modify the course of experimental rat leprosy in white rats. The drug does not appear to have any bacteriostatic action *in vivo* on Myco. leprae muris although *in vitro* it has been shown to have a bacteriostatic effect in a 1 in 1,000 dilution.

Epidemiological.

(a) *Leprosy Investigation Centre, Bankura.* A thorough re-survey of the area is under progress under the Leprosy Investigation Centre, Bankura. The actual leprosy survey has been preceded by a fresh census of the whole area, which has indicated that the population of the area shows little variation from the findings of 1937.

(b) *Epidemiological surveys of small selected areas.* During the last two years Dr. Santra of the Indian Council of the British Empire Leprosy Relief Association has carried out intensive leprosy surveys in small selected areas of almost all provinces in India. The findings of these surveys have confirmed the existence of regional variations in leprosy in different parts of India. The variations affect the incidence of the disease, type distribution, age distribution and the sex distribution of the cases. The variations are found not only in widely separated areas, but also in areas lying close to one another in the same district or subdivision. It is very difficult to assess the significance of these variations. One factor which appears to have a bearing on the differences observed is the attitude of the people towards leprosy, and the presence or absence of a custom of isolation of cases of leprosy. The areas investigated may roughly be divided into two groups (i) the areas in which there exists ostracism of cases of leprosy, and some sort of isolation is practised, and (ii) the areas in which there is little or no ostracism or attempts at isolation. It may be stated in general that the areas where there are some attempts at isolation, a high lepromatous rate is associated with low gross incidence and a low child rate. On the other hand, in the areas where no isolation is practised a lower lepromatous rate is associated with a high gross incidence and a higher child rate.

Bacteriological.

(a) *Sabauraud's medium.* Attempts have been made to cultivate the lepra bacilli on Sabauraud's medium, a medium used for growing fungi. No success has, however, been obtained.

(b) *Thiamine medium.* Recently one worker has reported the successful cultivation of leprosy bacilli on a medium containing thiamine, cholestrol, and rabbit serum. This work has been repeated by us but no indication has been obtained of the growth of bacilli in this medium.

22. Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras.

The work of the enquiry was continued under the following heads :—

1. Epidemiological and Preventive.
2. Clinical and Pathological.
3. Therapeutic.
4. Animal.

1. Epidemiological and preventive.

The investigation of certain selected villages in North Arcot which were taken for survey last year was continued, and figures have brought out interesting facts emphasising the importance of contact in the epidemiology of leprosy. Table I illustrates that the proximity of a village to one in which there is a heavy incidence appears to have no influence on the spread of leprosy in the less affected village.

Table I.

Villages with high incidence.		Distance of other villages in relation to heavy infected village.		
Name.	Incidence per 1000.	Name.	Distance.	Incidence per 1000.
Mottur	124.00	Kalambat	3 furlongs	Nil
Gummamkuppam	83.60	Gummamkuppam	50 yds.	51.8
		Anaikattiputhur	12 furs.	10.0
Kosavanputhur	43.8	Kosvânputhur cheri	20 yds.	Nil
		Pasumathur village	1½ ms.	16.2
		Pasumathur cheri	1½ ms.	8.3
		Netteri village	1¼ ms.	Nil
		Netteri cheri	1¼ ms.	1 neural case
		Vepur Vill. & cheri	2 ms.	Too low
Pallalakuppam cheri	73.40	Pallalakuppam village	1 fur.	23.2
		Yerrikuthi cheri	3 ms.	74.2
		Yerrikuthi village	3 ms.	4.6
		Chokarasikuppam	2 ms.	28.9
		Samarasikuppam	2 ms.	8.7
		Samarasikuppam cheri	2 m. 1 fur.	Nil
Asanambat (Malarial index 39.9%)	60.70	Asanambat cheri	100 yds	18.2
		Kallaparai (Malarial index 39.9 per cent)	1 ml.	16.0
Mittur (Chittoor Dist.)	120.55	Veerasettipalli	½ ms.	57.0

During the year further survey work has been undertaken in South Arcot, the figures are not yet complete for the purpose of analysis, but the introduction of leprosy into a village again seems to have no relationship to the incidence of the disease in the contiguous villages. One factor of importance which is illustrated both in South Arcot and in the above villages is that leprosy is frequently introduced in the first instance as a result of close marriage relationship between a heavily infected village and one in which the incidence is low or non-existent. We do not suggest that this is the result of any hereditary or family susceptibility but of the close contact which is established through marriage of infected cases with healthy members of the community. An epidemiological survey has been completed in another area in Saidapet and the table which follows gives a summary of the position.

TABLE II.

	M.	F.	Total
Total population (adult) ..	938	912	1850
Number examined (adult) ..	867	863	1730
Total population (children) ..	459	573	1032
Number examined (children) ..	430	529	959
Percentage of population examined ..	..	93.3%	
Gross incidence ..	Per 1000	30.86	
Child incidence ..	Per 1000	41.71	
Sex incidence—Male ..	Per 1000	38.55	
Sex incidence—Female ..	Per 1000	23.70	
Sex rate ..		60.24%	
Child rate ..		48.19%	
Open case rate ..		15.66%	

TABLE III.

Analysis of Cases by Type and Age.

Age group.	NEURAL. No.	NEURAL. percentage.	LEPROMATOUS. No.	LEPROMATOUS. percentage.	Total for age group.	Percentage of total cases.
0—14	36	90.0	4	10.0	40	48.2
15—34	17	85.0	3	15.0	20	24.1
Over 34	15	65.2	8	34.8	23	27.7
Total	68	81.9	15	18.1	83	100.0

It will be noticed that the child rate is high (48.19%) while the open case rate is low (15.66%). This may be explained by the fact that the population in two of the streets is very mobile and changes of tenants are frequent; under such circumstances it is very difficult to trace all open cases, for many of them may have moved from the area. An analysis of the age groups indicates an increased incidence in the lower age-groups, and suggests leprosy in this area, as in other areas of Saidapet proper, is a disease of serious endemicity.

In this connection it should be recorded that a resurvey of the Adidravida colony (Mambalam Village) of Saidapet which was started last year was completed. In this Harijan village all but one of the open cases have been isolated in the Lady Willingdon Leprosy Sanatorium.

It is interesting to note when comparing the incidence of leprosy in a group of villages*, all within a radius of five miles, that it varies from nil to 124 per thousand. On a closer analysis it is seen that the incidence does not seem to be affected by closeness of highly endemic villages, the presence of other diseases, e.g., malaria, or by economic conditions. The following points are stressed. The village of Mottur has an incidence of 124 per thousand and yet that of Kalambat three furlongs away has no leprosy. The introduction of leprosy into Mottur has been traced to a group of villages in Chittoor bordering N. Arcot. It is noted that the villages around Veerasettipalli have also a high incidence of leprosy. This group of villages maintains close marriage relations with Mottur, and the striking example is Kosavanputhur village with an incidence of 43.8 per thousand, whereas the Cheri less than 30 yards away has no leprosy, although the Cheri folk work in the caste village. It is stated that this village was also infected from the Chittoor District especially the Veerasettipalli group. Another interesting village is that of Asanambat where there is a high malarial incidence. Here the incidence of leprosy is 60 per thousand whereas in the Cheri, only 100 yards away, there are only four cases of leprosy. The evidence suggested that leprosy was introduced into Asanambat by Canarese shepherds and Lingayats who migrated from a group of villages in the Mysore State which is over 200 miles distant. This emphasises our contention that the greatest factor in the introduction and maintenance of leprosy is contact with open cases. Proximity of an infected village, poor economic conditions, or predisposing diseases seem to play little or no part in the spread of leprosy. It has been observed that malaria and leprosy are not frequently co-existent, but it is apparent from these figures that given contact with open cases leprosy will become established in a malarious village. Again, therefore, we would stress the importance of intensity of contact, for it appears that even in highly endemic areas not all villages present a leprosy problem, and only in certain ones it is necessary to undertake preventive measures. Such observations are of extreme importance in planning an anti-leprosy campaign.

Under this heading we have included the investigation into the methods of prevention at the rural centre established 23 miles from Chingleput. During the year a resurvey of the largest village, in the group has been completed and the following tables analysing the age-group incidence during the first five years is of interest.

TABLE IV.

Polambakkam Village—1939 Survey.

Analysis of Cases by Type and Age.

Age group.	Neural		Lepromatous		Total for age group.	Percentage of total cases.
	No.	percentage.	No.	percentage.		
0—14	10	32.26	0		10	32.26
15—34	3	9.67	4	12.90	7	22.57
Over 35	10	32.26	4	12.90	14	45.16
Total	23	74.19	8	25.80	31	100

* Vide table I.

TABLE V.

Polambakkam Village—1942 Survey.

Analysis of Cases by Type and Age.

Age group.	NEURAL		LEPROMATOUS		Total for age group.	Percentage of total cases.
	No.	percentage.	No.	percentage.		
0—14	13	30.23	—	—	13	30.23
15—34	9	20.93	6	13.95	15	34.88
Over 35	11	25.58	4	9.30	15	34.88
Total	33	76.74	10	23.25	43	100

TABLE VI.

Polambakkam Village—1944 Survey.

Analysis of Cases by Type and Age.

Age group.	NEURAL		LEPROMATOUS		Total for age group.	Percentage of total cases.
	No.	percentage.	No.	percentage.		
0—14	3	9.37	—	—	3	9.37
15—34	11	34.37	3	9.37	14	43.74
Over 35	11	34.37	4	12.50	15	46.87
Total	25	78.11	7	21.87	32	100

TABLE VII.

Polambakkam Cheri—1939 Survey.

Analysis of Cases by Type and Age.

Age group.	NEURAL		LEPROMATOUS		Total for age group.	Percentage of total cases.
	No.	percentage.	No.	percentage.		
0—14	4	19.04	—	—	4	19.04
15—34	3	14.29	3	14.29	6	28.58
Over 35	10	47.61	1	4.76	11	52.37
Total	17	80.94	4	19.05	21	100

TABLE VIII.

Polambakkam Cheri—1942 Survey.
Analysis of Cases by Type and Age.

Age group.	NEURAL No.	NEURAL percentage.	LEPROMATOUS No.	LEPROMATOUS percentage.	Total for age group.	Percentage of total cases.
0—14	5	22.72	—	—	5	22.72
15—34	5	22.72	2	9.09	7	31.81
Over 35	9	40.90	1	4.54	10	45.45
Total	19	86.36	3	13.63	22	100

TABLE IX.

Polambakkam Cheri—1944 Survey.
Analysis of Cases by Type and Age.

Age group.	NEURAL No.	NEURAL percentage.	LEPROMATOUS No.	LEPROMATOUS percentage.	Total for age group.	Percentage of total cases.
0—14	3	16.66	—	—	3	16.66
15—34	2	11.11	2	11.11	4	22.22
Over 35	10	55.55	1	5.55	11	61.10
Total	15	83.32	3	16.66	18	100

It is further of interest to note that the age incidence has decreased in the younger age groups with a corresponding rise in the older age groups, 32.26% in the 0–14 age-group has decreased to 9.37% in Polambakkam village between the years of 1939-1944 and in the Adidravida colony from 19.04% in 1939 to 16.66% in 1944. This has been accompanied by a fall in the gross incidence in the village from 52.89 to 44.13 per thousand, and in the Adidravida Colony from 44.00 to 38.79 per thousand in the last two years. We believe that these figures give some support to our belief that the policy of night segregation of open cases from contact with healthy persons, especially children, is bearing fruit. It is confidently hoped that during 1945 Government will take over this preventive experiment extending it to other villages in the area by declaring a group of eight villages as segregation areas under the Public Health Act (amended 1944) and thus establish the first organised unit for investigation into the control of leprosy in a rural area by night segregation of open cases.

2. CLINICAL AND HISTOPATHOLOGICAL INVESTIGATION.

It is our endeavour to correlate the clinical signs with the histopathological changes in the skin, for we believe, that the more attention is given to this the greater are the chances of finding effective therapeutic or other measures to deal

with the disease. Our general conclusions with regard to tissue reaction in leprosy are as follows :—

All reactions of the body to M. leprae are essentially defensive in nature. This defensive reaction can be divided into that which is effective and leads to the control and elimination of the disease, and that which is ineffective and results in the further dissemination of the M. leprae and consequent extension of the disease. The M. leprae has a particular predilection for cutaneous tissue, and if we look upon nerve tissue as embryologically an infolding of the primitive ectoblastic layer it can be understood why this should also be attacked. Our observation and researches have caused us to conclude that of the effective type of tissue reaction there are essentially two varieties:

(1) Fibrotic.

(2) Cellular.

The fibrotic reaction is seen particularly in the neural anaesthetic cases and to a lesser extent in the simple macular lesions with peripheral nerve involvement. In this variety fibrosis occurs early and produces extensive nerve damage, resulting probably in the destruction of the M. leprae in the nerve tissue and thus dealing effectively with the invading organism, but resulting in deformities.

The effective cellular reaction is well seen in the tuberculoid case where, as a result of the violent tissue reaction and the formation of tuberculoid foci, the invading M. leprae is anchored in the tissue and prevented from dissemination throughout the reticulo-endothelial system. In this variety nerves are also involved, but here it is not a fibrous tissue reaction which is first seen, but a cellular reaction which not infrequently results in caseation and nerve abscess formation. The recognition of these two varieties of reaction is particularly important, because in the fibrotic type operation is useless but, in the cellular type of reaction operation is the treatment of choice. This observation explains why certain workers state that operation is of little value while others say it is the procedure of choice. The decision to operate depends entirely on the nature of the tissue reaction.

Ineffective tissue reaction is seen in a lepromatous case where the response to the invasion with M. leprae is not one resulting in fibrosis or attempts at anchoring the bacilli, but a general phagocytosis by the wandering cells, (macrophages) resulting in wide-spread dissemination of the bacilli. This explains why lepromatous leprosy is so difficult to treat.

In between these two varieties of reaction occur the various cellular reactions which have to be styled, for the present as atypical. The varieties of cells mobilised are so great that it is impossible to summarise the changes in this large and complicated group which, as was stated last year is slowly being unravelled. Our general conclusions are that the various varieties of leprosy remain true to type and that it is only a small proportion of cases which pass into the lepromatous type, and, as stated previously, these are found in the simple macular and incipient varieties where the histological features are largely non-specific. We are of opinion through the observation of an increasing number of atypical or intermediate cases, that such cases generally remain true to type and do not as a rule pass into leproma. Our opinion with regard to leproma remains essentially the same that these pass through a prelepromatous stage—incipient or simple macular in children—suspicious or less frequently simple macular in adults. We have seen several cases among the adults which have been diagnosed as suspicious lesions with no definite signs of leprosy, apart from hypopigmentation, which several years later have become leproma. These observations are of particular importance as they have a very direct bearing on the question of prevention.

3. Therapeutic experiments.

Work has continued throughout the year on the trial of new remedies, but so far with completely inconclusive results. The experiments to discover the optimum dose for the hydnocarpus remedies has been pursued throughout the year. In previous experiments there appeared to be little difference in the results of treatment whether high dosages (above 15 c.c.) or low dosages (below 10 c.c.) were given. In this series, three groups were chosen ; (A) on subcutaneous injections alone, (B) on intradermal and subcutaneous, (C) on intradermal alone.

It will be seen from Table X that the number of cases which have become negative in the intradermal and subcutaneous group exceeds the number of negatives in those receiving intradermal injections or subcutaneous injections alone.

Table X.

Results of 3 groups of cases put on Intradermal, Subcutaneous & Intradermal, and Subcutaneous injections respectively after one year's treatment.

Negative.	Much improved.	Improved.	Stationary.	Worse.	Total.
		Intradermal Group.			
6	16	24	46	24	116
5.2%	13.8%	20.8%	39.7%	20.8%	..
		Subcutaneous and Intradermal.			
13	27	29	32	13	114
11.4%	24.6%	25.4%	28.1%	11.4%	..
		Subcutaneous Group.			
2	1	16	17	14	50
4%	2%	32%	34%	28%	..

This may indicate that to obtain the best in treatment it is necessary to use both the intradermal and subcutaneous methods. This opinion is strengthened as it is seen in Table XI, that the average length of treatment in the subcutaneous and intradermal groups was shorter than in the other two groups although the results were more satisfactory.

Table XI.

Average period of treatment.

	Subcutaneous and Intradermal group.	Intradermal group.	Subcutaneous group.
Negative	2 years 1 month	3 years 1 month	3 years 10 months
Much improved	1 year 10 months	2 years 2 months	2 years 8 months
Improved	1 year	1 year 6 months	2 years.

Therapeutic experiments in leprosy are extremely difficult, and before any definite conclusions can be drawn this experiment must be continued for a further period.

Leprosy and Syphilis. Muir (1927) stated that a positive Wassermann reaction in leprosy was most likely to be due to complicating syphilis. If this is the case then all patients with leprosy should show an improvement in the W. R. reaction on treatment for syphilis. The treatment used for convenience was that recommended by Muir (Hg. 33). Ten cases were chosen as a preliminary experiment : five were treated with Hg. 33, and five left as controls. In the control group two cases, both with strongly positive Wasserman and Kahn reactions, became negative and in the treatment group none. The negative result corresponded with marked improvement in the clinical and bacteriological condition and suggests that leprosy was the factor which caused the positive reaction. A further analysis of the patients with positive serum reactions* was undertaken and Table XII gives the percentage of positive reactions in the various types in the Lady Willingdon Leprosy Sanatorium.

TABLE XII.

Type.	Kahn and W.R. Positive.	Kahn and W.R. doubtful.	Kahn and W.R. negative.	Kahn and W.R. total cases.	Percentage of W.R. positive.
L3	14	4	16	34	41.0
L2	36	20	109	165	21.0
L1	24	14	161	199	12.0
Neural of all degrees	1	1	20	22	4.5

It will be seen that the positive rate decreases markedly from 41% in the advanced leproma to only 4.5% in the Neural group. Further, in an analysis of 14 lepromatous cases which were discharged from the institution, four showed a strongly positive Wassermann on admission and when discharged the Wassermann reaction was found to be negative, although no anti-syphilitic treatment had been given.

In our opinion these results give strong support to the theory that a positive Wasserman or Kahn may have no significance in leprosy, and that physicians are not justified in giving anti-syphilitic remedies in leprosy unless there are clinical signs of past or present syphilis, or a history of infection.

These figures confirm our tentative opinion of last year and alter our whole outlook on the question of leprosy and syphilis.

4. ANIMAL EXPERIMENTATION.

This was continued throughout the year. In one instance a lesion in a monkey has been produced which showed definite tuberculoid structure; this was of particular interest and as the lesion occurred on the forehead a place where the animal had never received any previous inoculation. In several other instances epithelioid and giant cell reaction was produced after

* 75 per cent of the serum reactions were Kahn's antigen and the remaining 25 per cent Wassermann.

intracutaneous inoculation of bacilli. It is hoped that the results of the last several years experiments will be published in due course for several interesting observations have been indicated.

Lepromin reaction. The work on lepromin has been continued on a reduced scale owing to difficulties in preparing the refined lepromin. The following table confirms our previous observation that all lepromatous cases are negative and all tuberculoid cases are positive, whereas the atypical or intermediate group are generally negative but may be mildly positive.

TABLE XIII.

Classification.	Negative.	+	++	+++	Total.
L1	168	..	..	..	168
Tbd.	..	2	4	2	8
Intermediate	25	..	..	..	25
Neural	10	1	..	..	11
Total	203	3	4	2	212

PLAGUE.

23. Plague Researches under the Director, Haffkine Institute, Bombay.

1. Field trials of sulphathiazole and sulphadiazine.

A plague epidemic at Poona during the latter part of 1943 gave an opportunity to conduct a trial into the comparative value of sulphathiazole and sulphadiazine in the treatment of plague. The trial was carried out in the Infectious Diseases Hospital at Poona and lasted from the middle of November 1943 to the middle of February 1944. During this period, 182 cases of plague were treated

Treatment and dosage. Admissions were treated in strict rotation with sulphathiazole and sulphadiazine, no selection of cases being made. On admission, 0.5 c. c. of blood of each patient was drawn and plated on two agar slopes to determine the presence or absence of septicaemia and its degree at the time of admission. In the absence of septicaemia, bubo was also punctured and sucked up material plated in clinically doubtful cases.

In the first 46 sulphathiazole cases, the drug was given in a dosage of 10 g. the first day and 6 g. a day for the subsequent 7–10 days. Subsequent sulphathiazole cases received 12–14 g. the first day and 6–9 g. a day on subsequent days. The level of free sulphathiazole attained in both groups of cases was 5–10 mgm. per 100 ml.

Sulphadiazine was given in dosage of 10 g. the first day and 6 g. a day on subsequent days. The level of free sulphadiazine attained in the blood with this dosage was 10–15 mgm. per 100 ml. In a few very serious cases which received 14 g. the first day and 6–9 g. a day subsequently, the level of the free drug attained was 15–20 mgm. per 100 ml.

Results. From 182 plague cases treated, 26 cases are excluded from reckoning—24 moribund cases that died within 24 hours of admission and 2 that left the hospital against medical advice. For controls, the cases treated in some previous trials with iodine are given here for comparison—the control cases which died within 24 hours of admission are similarly omitted.

All cases of Bubonic Plague treated.

Drug.	Number of cases.	Number of deaths.	Case mortality per cent.
Sulphathiazole.	75	16	21.3
Sulphadiazine.	81	10	12.3
Controls-given the usual hospital treatment, i.e., iodine intravenously.	149	80	53.6

Since the presence or absence of septicaemia at the commencement of treatment is the one important factor which decides the issue, the results of the treatment in septicaemic cases are given separately.

Case with plague septicaemia at the commencement of treatment.

Drug.	Number of cases.	Number of deaths.	Case mortality per cent.
Sulphathiazole	40	15	37.5
Sulphadiazine	43	9	20.9
Controls—i.e. iodine intravenously	75	68	91.0

The results of sulphadiazine appear to be slightly superior to those of sulphathiazole but statistically the difference is not significant. No serious toxic reactions were encountered in cases treated with either of the two drugs. Even the incidence of mild reactions was low. However, sulphadiazine, on the whole, produced fewer toxic reactions.

II. Plague Vaccine.

1. *A new broth vaccine.* During the year we have been working on a new broth vaccine. The main features are as follows :—

(a) *Medium.* Acid hydrolysate of casein of Muller and Johnson (J. Immunology. 1941, 40, 33). 10 kg. of casein are suspended in 84 litres of water and 3.2 litres of HCl (S.G 1.16) and 4.8 litres of H_2SO_4 (S.G 1.84) are added and the total volume brought up to 100 litres. This is refluxed for 72 hours with steam, mixture maintaining a temperature of 99° to 100°C. H_2SO_4 is neutralised with $BaCO_3$, filtered and the filtrate diluted to have a nitrogen content of 270 mg. for 100 c.c. Then HCl is neutralised with NaOH and pH adjusted to 7.2 ; and sterilized and dispensed in 1 litre quantities in modified Haffkine flasks. The medium so prepared does not contain proteins, and does not give biuret reaction. This is the simplest form of Muller and Johnson casein hydrolysate and we find that it gives very good growth of *Pasteurella pestis.*

(b) *Inoculum.* Fully virulent strain of *P. pestis* grown in broth for 3 days, about 5 c.c. per litre.

(c) *Incubation (temperature and duration).* Flasks are incubated at 28°C. for 2 weeks.

(d) *Addition of formalin and phenyl mercuric nitrate.* After the purity of growth in each flask has been tested 10 c.c. of 1/10 dilution of 40% formalin is added to each flask to make a strength of 0.1 per cent. Flasks are then incubated at 37°C. for 7 days. Then 20 c.c. of 50 mg. per 100 c.c. solution of phenyl mercuric nitrate is added to each flask, i.e. 1 mg. per 100 c.c. of the salt.

It will be noted that formalin is used to detoxicate the vaccine and phenyl mercuric nitrate is used as the preservative instead of phenol.

2. *Protective power of the new vaccine compared with agar vaccine.*

		Mouse Protection Dose.
(1)	New broth vaccine	0.003 c.c.
(2)	Agar vaccine grown at 37°C. heat-killed and carbolised (0.5%)	0.004 c.c.
(3)	Haffkine vaccine	0.006 c.c.

3. *Toxicity.* Measured as the dose killing 50 per cent. of the mice used.

(1)	New broth vaccine	0.8 c.c.
(2)	Agar vaccine	0.8 c.c.
(3)	Haffkine vaccine	0.2 c.c.

4. *Keeping qualities of the vaccines.*

Ampouled vaccines were stored at 37°C. and one batch tested after 8 weeks. The effect of longer storage is still under investigation.

		Mouse Protection Dose Original.	Mouse Protection Dose after 8 weeks storage at 37°C.
(1)	New broth vaccine	0.003	0.003
(2)	Agar vaccine	0.004	0.006

The results reported here indicate that in the new broth vaccine we have a plague vaccine as potent as an agar vaccine, if not better, is no more toxic and has the advantage of better keeping qualities. This last factor is of particular value in a tropical country like India.

24. Plague enquiry in the Salem District under the Director of Public Health, Madras.

Plague enquiry at Hosur comprised of the study of the prevailing rodents and their fleas contributing to the endemicity of bubonic plague in Hosur, the application of cyanogas in plague control and the treatment of plague with sulphathiazole.

X. brasiliensis, X. cheopis, X. astia are the prevailing fleas on domestic rodents, the former predominating through all seasons. The *brasiliensis* index for *R. rattus* was lowest in June (1.3) and highest in November (3.9) while that of cheopis was lowest in February (1.0) and highest in August (2.8). X. astia was however almost the exclusive flea on field rodents as has been our experience in all other areas in this Province.

Four hundred and sixty-eight wild rodents were examined during the year for studies in sylvatic plague. 3 gerbills from the same burrow were found infected. The fleas recovered from them also were infected. These gerbills were obtained from the vicinity of the infected village. Examination of field rodents and their fleas away from villages is being continued to see how far infection has extended among field rodent colonies in this area.

Study of rodent nests was continued. 124 nests of *R. rattus* yielding 3109 fleas showed that brasiliensis, cheopis and astia prevailed in the ratio of 1.0 : 1.01 : 0.14 respectively in ground burrows and 1.0 : 0.42 : 0.07 respectively in nests from the ceiling of houses. 6 gerbil nests with about 200 fleas consisted of 93% astia and the remaining *brasiliensis* and *cheopis*. 37 squirrel nests with 2767 fleas comprised of 99% ceratophylus fleas. These observations show the preferential environmental conditions favouring the numerical prevalence of the respective fleas.

Vigorous cyanogas fumigation was carried out in all the villages in the experimental area. There was noticeable reduction in the incidence of plague in spite of the somewhat restricted application of cyanogas due to shortage of supplies.

Five hundred and sixty four plague cases were treated with sulphathiazole and allied group of drugs. 194 cases were treated with sulphathiazole only and 129 of them recovered giving recovery rate of 71%. Results were most striking where 25–50 gms. of the drug were administered.

PHARMACOLOGY.

25. Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.

1. *Daemia extensa.*—This plant, known as 'Chagalbanti' in Bengal, is a reputed drug in the indigenous system. It contains a number of bitter substances some of which are active and some are inactive. The non-toxic physiologically active bitter substance has been found to raise the blood pressure in spinal and decerebrated cats and after atropinisation, vagus separation, and ergotinisation. It has a marked stimulating action on the intestine, bladder and uterus. The drug appears to be well tolerated by frogs, rats, and mice but comparatively less so by cats. From the results so far obtained the drug appears to be very promising. The results will be shortly published.

2. *Kamala powder.*—This is a colouring agent obtained from Mallotus philippinensis. A study of the toxicity of the powder was undertaken with a view to its utilisation for human consumption. Batches of guinea-pigs were fed with the powder (dissolved in 'Vanaspati') in different doses and for varying lengths of time. The animals were then killed and the vital organs (e.g. spleen, liver etc.) removed, fixed and stained. It is seen that the powder when fed to guinea-pigs in varying doses of upto 50 mg/kg daily for a period of four months does not seem to produce any toxic effect on these animals. The colouring material may be utilised for human consumption without any deleterious effects.

3. *Alstonia scholaris.*—Total alkaloids of Alstonia scholaris in combination with small amounts of quinine were tried in several cases of malaria with a view to save quinine in mass treatment of malaria. The results do not confirm the opinion that alkaloids of Alstonia scholaris substantially reduce the amount of quinine that would otherwise be necessery for a cure.

4. *Rauwolfia serpentina.*—Resins from different varieties have been quantitatively isolated for comparative study. They have been further fractionated by different solvents. The resin content of the Dehra Dun, Bengal, Bihar, Assam varieties are 1.5 per cent, 1.14 per cent. 1.25 per cent. 1.8 per cent respectively.

5. *Terminalis chebula.*—Two varieties available in the bazaar were chemically examined. The common variety contains 38 per cent tannic acid and tannins, 2.68 per cent resin, and .52 per cent of anthraquinone derivatives. The 'jungli' variety (small size) contains 57.8 per cent of tannic acid and tannins, 3.66 per cent of resin, and .88 per cent anthraquinone derivatives. Animal experiments with different fractions are in progress.

6. *Achyranthes aspera.*—The plant contains nitrates but does not give any test for alkaloid or glucoside. Animal experiments did not reveal the presence of any other therapeutically active principle.

7. *Roylea elegans.*—The plant contains a bitter principle and an alkaloid. In a dilution of 1 in 4000 it has got no effect on paramoecia. A decoction of the leaves was tried on 2 malaria patients. The results are not promising.

8. Four samples of *Artemesia* from Baluchistan have been examined for their santonin content which was found to be present in traces in one sample only.

9. Seeds, pods and leaves of *Cassia tora* and *Cassia occidentalis* have been separately examined for their anthraquinone content with a view to their use as anthracene purgatives. The percentage of anthraquinone in Cassia tora seeds and pods are 5.38 and 5.2 respectively, the leaves contain none. The seeds and pods of Cassia occidentalis contain 5.9 per cent and 5.3 per cent of anthraquinone, the leaves containing a trace only.

10. *Terminalia bellerica.*—It is one of the ingredients of 'triphala', used extensively in the indigenous system for its purgative properties. From preliminary investigation it appears that the drug has a slight tonic effect on the heart and a specific effect on the spleen. Isolation of the active principle is in progress.

11. *Chemotherapeutic studies in malaria.*—A preliminary study of the following drugs is in progress on ambulatory patients from the outdoor who show malarial parasites in blood. It is intended that those drugs which show any encouraging results would be taken up for further study under controlled conditions on indoor patients. A great difficulty lies in the fact that the majority of the patients fall off in the course of treatment and afterwards. The results noted below are tentative.

(a) *Neo-antifebris.*—It is claimed to be a substitute for quinine and was sent by the Surgeon-General with the Government of Bengal for investigation. Of the 10 outdoor patients in which the drug was tried 3 only turned up for treatment for a period of one week which was not adequate. In these three, however, the drug produced an apparent cure. Further trial could not be given for want of sufficient supply of the drug.

(b) *Qureen.*—A remedy for malaria was sent by the Surgeon-General with the Government of Bengal for investigation. It is prepared from indigenous drugs. The drug was given to five outdoor patients. In 3 cases it proved a failure and in 2 cases it produced an apparent cure. Further trial is in progress.

(c) *Neoquin.*—The drug was administered to 10 outdoor patients. The fever responded to treatment in the course of a week. In 4 cases which could be followed up relapses were reported. Further trials are in progress.

(d) *Quinarsol.* It is a combination of basic quinine hydrochloride with sodium dimethyl arsenic acid and phenyl dimethyl pyrazolone. The quantity of the drug supplied was very small, hence it could not be tried on more than 4 patients of whom three fell off on the second day. The remaining one received intensive treatment of three course which even, did not prove to be effective.

Anti-tertian pills. The drug is prepared from indigenous drugs and contains 1 grm. of quinine per tablet the total dose recommended being 12 tablets for each adult patient. It has been tried in 15 outdoor patients. The fever generally responded to treatment within 5 days but in many cases relapses have been reported. Further trials are in progress.

26. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S., at the Drug Research Laboratory, Jammu, Kashmir.

INULA ROYLEANA DC. (*Vern. mullun*)

The work regarding the chemistry and pharmacological action of *Inula-royleana* was completed and a paper embodying the results of these studies was forwarded for publication in the Indian Journal of Medical Research.

Lippia nodiflora Rich. (Vern. Bukhan)

This plant grows throughout India and is very widely used in indigenous medicine as a febrifuge and diuretic. The plant was successively extracted with petroleum ether, ether sulphuric, chloroform, ethyl acetate and alcohol. Further examination of the residues after removing the solvents showed the presence of a *'bitter substance'* in *the ethyl acetate extract* and a large quantity of *potassium nitrate in the alcoholic* extract. Large quantities of the drug were then taken up to obtain the active principle in a purer form from the ethyl acetate extract. It was found to occur in very minute quantities. From the very small yield of the bitter substance it would appear that the drug cannot possess any marked degree of therapeutic activity. The presence of large amount of potassium nitrate may well account for the alleged diuretic action of the plant.

Xanthium strumarium Linn. (Chotta Gokhuru)

This is an important plant being widely used both in Tibbi and Ayurvedic medicine as a diaphoretic and sedative. Whereas it is used as a pot-herb in Assam, the plant is considered to be poisonous to the live stock in Australia and America. The active principle of the plant which is a water-soluble glucoside was extracted from the endosperm of the seeds obtained by pressing out the fruit. The seeds contain 32% of a fixed oil in addition to the amorphous glucoside. Administration of as much as 30 to 40 mgm. of the active principle per kilo did not produce any effect either on respiration or blood pressure when administered intravenously to cats and rabbits. Average sized rabbits survived without effect on respiration and circulation even after the total administration of 120 mgm. of the substance. The active principle, therefore, appears to be physiologically not very active.

Verbena officianalis Linn. (Vern. Karaita)

This plant is widey used in indigenous medicine for multifarious purposes e.g. as a febrifuge, tonic and as a rubefacient in rheumatism. The whole plant was powdered and used for the isolation of the active principle. A glucoside has been isolated. This substance is not toxic to paramoecia and has no action when administered to experimental animals except a short and transitory fall of blood pressure following intravenous administration of large amounts.

Chrysanthemum cinerarifolium Vis.

Work on pyrethrum was continued with a view to study the effect of different methods of drying on the pyrethrin content of the flower-heads. For this purpose pyrethrum flowers were dried under different conditions i.e. throughout in the sun, throughout in shade, partially in the sun and partially in shade. Twelve samples of the flower-heads sent by the Forest Department, Kashmir, were analysed and it has been found that highest yield of pyrethrins was obtained in the case of flowers dried partially in the sun and partially in shade. The shade-dried samples have given comparatively low results while the sun dried ones gave little higher results.

Investigation on essential oil-bearing plants.

There is at present a large demand for essential oils both for medicinal and industrial purposes. A large number of essential oil bearing plants grow

wild in Kashmir State. A preliminary investigation of some of these has been carried out with the following results :

Mentha sylvestris Linn.—The plant was collected from two areas i.e. Jammu and Kashmir provinces. Physical constants of the oils obtained by the steam distillation of the plants were found to be identical. No menthol could be separated even after allowing the oil to stand for a week. The results of fractional distillation also showed that the plant contains very little menthol. The oil could, however, be used as a cheap flavouring agent due to its minty odour.

Lavender.—A sample of the flowers of the plant was sent to us by the Forest Department of Jammu & Kashmir State which has been analysed with the following results:

Yield of the oil 2.4 per cent, ester percentage 24.8 per cent, specific gravity 0.9128. Further study of the oil is being continued.

A preliminary survey of some other essential oil-bearing plants has also been done with the following results :

1. *Artemisia brevifolia*—0.3 per cent, 2. *Artimesia absinthium*—0.19 per cent. 3. *Juniper berries*—1.02 per cent. Detailed investigation of these plants is in progress.

The enquiry closely colloborated in the following work of Medicinal Plants and Food Poisons Enquiry under Imperial Council of Agricultural Research.

Monograph on the poisonous plants of India.

Owing to pressure of work in connexion with war and scarcity of paper, the Government of India suspended the publication of this monograph at a time when everything was ready to bring out the 1st volume of 800 pages. The Government have now instructed the press at Calcutta to print off volume I. This work is now in hand and it is hoped that the 1st volume will be published early in 1945.

Publication of papers.

The following 3 papers are nearing completion and will be published in the near future. (1) " Indian Plants liable to Produce Skin Eruptions " (2) " Poisonous seeds of India ", (3) " Reputed Abortifacient Plants of India ".

Survey of medicinal and poisonous plants of India.

Indian medicinal plants have been grouped under the following 4 categories. (a) Medicinal plants used in the Western Pharmacopoeias and their substitutes growing in India (b) Plants used in Western medicine and recognized in the extra Pharmacopoeias (c) Important plants used in indigenous medicine (d) Plants with alleged medicinal properties.

From the vast data that have been gathered from herbaria in India and through extensive work in the field, the exact distribution for groups (a) and (b) is being worked out. The work under (a) has been practically completed and is now being got ready for publication in the form of a monograph entitled " Distribution of the British Pharmacopoeial Drugs of vegetable origin and their substitutes growing in India with exact localities of actual collection ". A number of substitutes have been described for drugs not available in India and for those which

grow scarcely in their natural state. The exact localities of those plants which are found in India as well as of their substitutes have been given. It is hoped that the information collected will be of great value in harnessing the indigenous resources of the country at a time when foreign supplies are greatly reduced.

HERBARIUM.

There are now about 8,000 specimens representing about 1,700 species of the medicinal and poisonous plants of India in the herbarium. About 300 more species are in the course of being collected.

27. Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

SYNTHETIC ANTIMALARIAL DRUGS.

Three new drugs were investigated for their antimalarial activity in rhesus monkeys infected with *Plasmodium knowlesi*. They were (i) Formaline Sulphonilamide (ii) Formaline Sulphathiazole and (iii) N'—methyl thiazoline. The drugs were administered orally in doses varying from 0.5 G. given once to 0.5 G. given twice a day for three consecutive days. All the three drugs showed antimalarial property but in all the three cases this property was inferior to that of Sulphathiazole, administered similarly in comparable doses. The two formaline compounds were poorly absorbed, the highest concentration in the blood obtained after the largest dose employed being of the order of about 2.1 mg. per 100 ml. It was observed during the experiments that these two drugs were partially hydrolysed in the gastro-intestinal tract liberating small quantities of formaline.

The thiazoline compound was better absorbed than the formaline compounds, but was found to be more toxic. The antimalarial activity of this compound was about the same as that of the formaline compounds.

IMMUNITY IN MONKEY MALARIA.

Two large size (more than 15 Kg.) monkeys whose identity could unfortunately not be determined were found to be immune to *P. knowlesi* infection. A third similar monkey was also therfore taken to be immune to *P. knowlesi* infection. The blood of this monkey was found to match perfectly with the blood of ordinary rhesus monkeys which are susceptible to knowlesi infection. The blood of a susceptible monkey was then replaced by the blood of the immune monkey to an extent of about 80% of its total blood volume and the susceptible monkey infected intraperitoneally with *P. knowlesi*, immediately afterwards. It was found that the monkey developed the infection and died of it in the usual way. This experiment showed that the immunity in the large size monkey was 'cellular' and not 'humoral'. The enquiry has been discontinued from October, 1944.

OTHER RESEARCHES.

28. Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

In continuation of the previous work the role of quinine in the prevention of malarial transmission through stored blood was studied in a series of twelve rhesus monkeys. This study was conducted in three different ways.

In the first experiment, samples of blood, obtained from P. knowlesi infected monkey, were kept in the refrigerator at 4°C for periods varying from 3 to 7 days and then transfused into 8 animals which acted as recipients. In 5 of these, the transfused blood was previously treated with quinine, in concentration of 1 in 500, while in the remaining three no such treatment was given. Since none of the five animals, transfused with quinine-treated blood was infected, while all the other 3 animals were infected, it may be reasonably concluded that quinine, as contrasted with soluseptasine and Dagenan was effective in preventing transmission of malaria through transfusion of malaria-infected blood

In the next experiment, attempts were made to determine the lowest concentration of the drug in the stored blood which will prevent transmission of malaria. To determine the concentration of quinine required, two sample of parasitised blood which were stored and chilled at 4°C for five days and to which quinine had previously been added in strength of 1 in 1000 and 1 in 10000, respectively, were transfused in two monkeys ; while a third one, which served as a control, had no quinine added to the inoculum injected. These monkeys are still under observation so that no final conclusion is possible at present.

In the third experiment, attempts were made to sterilise an infected donor monkey. Repeated quinine injections were given so as to reduce the parasite rate to less than 1 per cent of red cells ; and the blood from such a monkey was transfused into another, serving as a recipient. Although the recipient monkey did not show any infection even after a month, the experiment needs to be repeated to have the conclusion thoroughly confirmed.

In addition to these experiments, a series of six other experiments were undertaken on four monkeys to study the effects of M & B 693 on the haemopoietic system of monkeys in relation to the concentration of the drug in the blood of these animals. The drug, as before, was given on an empty stomach, suspended in water and the dose varied from 0.125 gm./kgm. to 0.5 gm./kgm. in each experiment, lasting generally for about a week. During the experimental period, the blood picture was followed by almost daily blood count and the concentration of the drug was estimated by the Biological Standardisation Laboratory.

Of the four monkeys used, two succumbed—one, after receiving four daily doses of 0.5 gm./kgm. while the other, after receiving the full course of 0.5 gm./kgm. In the first, there was no evidence of anaemia at all but in the second, the haemoglobin and the red cells fell from 14.44 gms. and 5.51 millions to 11.0 gms. and 4.37 millions, respectively. These drops were associated with the maximum concentration of 12.7 mg. of the drug per 100 cc. of blood. Each of the two other monkeys received two full courses at an interval of a month—one received 0.3 gm./kgm. on the first occasion and 0.06 gm./kgm. on the second occasion ; while the other received 0.5 gm./kgm. and 0.125 gm./kg,. on two

different occasions. In both these monkeys haemoglobin and red cells were, as a rule, inversely related to the concentration of the drug in the blood; in the first, the lowest level of hamoglobin reached was 10.75 gms. and this was associated with 3.6 mgm. of the drug per 100 c.c. of blood but in the second, the corresponding minimum value of 10.18 gm. was reached two days after the last dose had been given when presumably the blood was free from the drug. It will thus be seen that while in the same animal, the blood values varied more or less inversely with the concentration of the drug, there was no uniformity in the haematological reactions of different animals even with the same concentration of the drug in the blood.

Further experiments were designed to produce anaemia experimentally by causing liver damage through arsphenamine in five healthy rhesus monkeys weighing from 1 to 3 kgm. In two of these the initial dose of the drug given was 0.03 gm./kgm. increased by 0.01 gm./kgm. from week to week; one of these animals died after receiving two injections only, while the other received the full course of four injections. In neither, however, was there any evidence of anaemia as shown by repeated blood counts done at intervals of two to three days. Of the three other animals, one received two injections of 0.1 gm./kgm. and succumbed; in the remaining two which survived, four injections of 0.05 gm./kgm. each were given. In none of these animals, was there any significant drop in the haemoglobin and red cell levels. These results indicate that either the liver and the haemopoietic system of monkeys are more resistant to arsphenamine poisoning than those of dogs or that the brand of arsphenamine, used here, was less toxic than that used by workers experimenting with dogs.

29. Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

I. Studies of ringworm.

During this period 983 cases of ringworm infection of the body, nails, interdigital spaces and hair follicles were studied. The results are briefly stated below :—

Clinical varieties —

Ringworm of the body	.. ..	453
„ „ body & nails	.. ..	94
„ „ nails only	.. ..	53
„ „ toes and interdigital spaces	..	257
Ringworm of the hair—		
Sycosis barbae (tinea)	.. ..	34
Pustular folliculitis (tinea)	..	92

Racial incidence :

Indians	.. ..	786
European & others	.. ..	197

Seasonal influence — January to March — average 30 cases per month

April and May	„	90	„	„	„
June to September	„	180	„	„	„
October to December	„	74	„	„	„

As is expected the incidence is highest in summer and increases with the onset of the monsoon when the humidity is high. The figures for the cold weather are probably a continuation of the summer infection.

The causal organisms were determined by microscopic and cultural methods.

	Total.	Microscopic.		Cultural.	
		Positive	Negative	Positive	Negative
Ringworm of the body and nails ..	547	511	36	524	23
,, ,, nails only ..	53	42	11	48	5
,, ,, toes and interdigital spaces ..	257	196	61	237	20
Ringworm of hair (Sycosis barbae & Pustular folliculitis ..	126	0	126	21	105

Mycological findings on culture :

	Total Positive.	*Epid. floccosum.* (with variants)	*Epid. rubeum.* (with variants)	Tricho-phyton.	Monilia.
Body ..	524	362	43	119	..
Nails ..	48	20	4	8	16
Toes & interdigital spaces ..	237	109	24	63	41
T. barbae etc. ..	21	0	0	21	0

From the above it is apparent that *E. floccosum* is the most prevalent fungus causing ringworm of the body and nails. Trichophyton infection is also responsible for quite a large number of cases, being about 25 per cent of the total number of cases. The incidence of trichophytosis in India has not been stressed before.

II. Preventive measures.

Some of the drugs were selected and tested as regards their fungicidal properties against *E. floccosum* that being the prevalent organism. The drugs which had a good fungicidal property in a fairly high dilution were put to clinical test

Drugs tested :—

Maximum dilution which proved fungicidal when treated for 1 minute and 10 minutes respectively.

Watery solution of	1 minute		10 minutes	
Acetic acid glacial ..	1 in 1000	dilution.	1 in 2000	dilution.
Salicylic acid ..	1 in 500	,,	1 in 500	,,
Benzoic acid ..	1 in 500	,,	1 in 500	,,
Thymol ..	1 in 200	,,	1 in 500	,,
Formaline ..	1 in 200	,,	1 in 500	,,
Tr. Iodine ..	1 in 100	,,	1 in 100	,,
Tartaric acid ..	1 in 10	,,	1 in 100	,,
25 per cent alcoholic solution of				
Acetic acid glacial ..	1 in 4000	,,	1 in 10,000	,,
Salicylic acid ..	1 in 2000	,,	1 in 4000	,,
Benzoic acid ..	1 in 2000	,,	1 in 3000	,,
Thymol ..	1 in 1000	,,	1 in 3000	,,
Tartaric acid ..	1 in 1000	,,	1 in 2000	,,

Clinical trial :—

Clinical trial was given to the drugs which seemed to be good fungicidal in the laboratory tests (in vitro). These drugs were painted with a cotton swab twice daily after the usual bath or in the morning and evening on the parts of the body which are the common sites of the infection e.g. between toes, in the groin and inner sides of the thighs. Cases selected were those who usually get ringworm every hot weather in these places or who have been recently relieved of all symptoms of the infection but cure is doubtful and also who have infection in some other parts from where the infection is likely to spread to these areas.

The results in brief are :

Two per cent solution of glacial acetic acid in 25 per cent alcohol has proved to be the best of all the drugs so treated ; it is fairly reliable as a prophylactic against ringworm infection if used regularly during the hot weather and monsoon months. The drug is non-irritant to the skin and non-corrosive and non-staining.

Two per cent solution of salicylic acid or benzoic acid in 25 per cent alcohol can be used as a prophylactic but they are sometimes irritating to the skin and having keratolytic properties exfoliation and consequent damage to the skin might occur if used for a long time. Thymol in the same strength is less effective and more costly.

30. Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay.

Last year a study on the Schneider test was reported. This year a study of the Crampton test was undertaken.

The Crampton Test.

This is also called 'the blood ptosis test'. In this test the reactions of the pulse rate and the systolic pressure to the postural change of the body from the recumbent to the standing are taken into account. From these Crampton has worked out a percentage scale of physical fitness in the form of a table by adjusting the changes in pulse rate in terms of corresponding blood pressure variations. 50 is considered as the minimum score of fitness for males.

This test was done on 495 subjects comprising 418 males and 77 females. To see whether the test can grade the physical fitness of an individual the males were divided under three groups viz. (1) 'no exercise' group of those who never took any regular exercise, (2) 'moderate exercise' group of those who had the habit of taking some type of exercise such as walking or playing outdoor games daily and (3) 'athletic' group of those who were regularly taking athletic exercises in gymnasia. It is assumed that persons taking some regular exercise are physically fitter than those taking no exercise. The females were also divided into two groups 'no exercise' and 'moderate exercise'. There were no 'athletes' among them.

The mean scores obtained by these different groups are given in Table I.

TABLE I.—MEAN SCORES OF FITNESS BY CRAMPTON TEST.

	'no exercise' group		'moderate exercise' group		'athletic' group	
	males	females	males	females	males	females
Mean score with p.e.	70.43 ±.52	67.93 ±0.97	73.10 ±0.65	74.61 ±1.57	77.93 ±1.24	..
Standard deviation	12.15	11	11.20	9.87	10.85	..
No. of subjects in group	249	58	134	19	35	..

The influence of the following factors on the test was then studied.

Influence of exercise.—The difference in mean scores between the 'no exercise' and 'moderate exercise' and between the 'moderate exercise' and the 'athletic' groups is statistically significant. It may therefore be said that the test does grade physical fitness fairly well assuming that regular exercise does improve the fitness of an individual.

The 'significance' of these differences however vanished in most cases on comparing the mean scores of subjects correspondingly grouped to keep their diet, the time of day at which the test was done, and the diet as well as the time of day constant separately. This may suggest that the test in order to be of value may have to be done under standardised conditions.

Influence of sex—The corresponding male and female normal 'no exercise' and 'moderate exercise' groups could not be significantly differentiated from one another suggesting that sex probably does not influence the test.

Influence of diet.—Subgroups belonging to the same exercise group but having different diets (vegetarian and non-vegetarian) could not be differentiated significantly. This possibly means that the diet has no influence on the test.

Influence of community.—Subgroups Gujarathis, Deccanis, Parsis, Muslims etc. from the same exercise group could not be differentiated significantly by their mean scores. This suggests that the community influence on the test is probably nil.

Influence of time of day.—This was studied in a group of 23 subjects on whom the test was done in the morning as well as in the afternoon keeping the same time interval between the test and the previous meal. Their mean scores under these two conditions were not significantly different. This may mean that the time of day also probably does not affect the test.

On comparing the above results with those obtained with the Schneider test reported before it seems between the two the latter is a better test in grading the physical fitness of an individual than the former.

31. Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta.

The work carried out during the year 1944 may be dealt with under the following heads :—

Field work.

During this year, field surveys were carried out at Jhargram, a sub-divisional town in the district of Midnapore (West Bengal), Krishnagar, the head quarters of the district of Nadia, Chandranagar (Hooghly District) and Ratanpur in Bilaspur district (C.P.)

Jhargram is a small sub-divisional town in the district of Midnapore (West Bengal) situated on the main line of communication—B. N. Railway, Howrah to Nagpur C.P.—on the Western border of the district, at an elevation of 250 ft. above sea-level. The total population of the town is about 4,000, the new and well laid-out extension of the town being a colony of retired officials from the adjoining districts, while the indigenous population is mostly of Santals and other aboriginal tribes. The soil is entirely lateritic and the area is covered with *sal* jungle. The water-supply is from draw-wells which are fairly deep. There is no drainage system in the town.

Although the number of culicine mosquitoes is comparatively smaller than in other crowded towns, they were found to breed in sumps in the gardens adjoining cottages. *Culex fatigans* was found to be prevalent in the area.

Jhargram is due west of Midnapore and is within 20 miles from it. Records of temperature and humidity are not available for this town.

No case of filarial infection was detected in this area.

In this connection it is interesting to note that in the district of Midnapore which has a filarial index of 16 per cent, the infection is heavier in towns lying on the coastal belt (Contai and Tamluk), moderate in those situated inland (Kharagpur and Narayangarh) while no infection is found in towns lying at an elevated region in the north and west of the district (Chandrakona and Jhargram).

Krishnagar (Nadia District) in Bengal has a microfilaria-rate of 2.1 per cent (3 persons positive for microfilariae out of 139). The infection is solely due to *Wuchereria bancrofti*. Culicine mosquitoes especially *Culex fatigans* were found to breed here in fairly large numbers. Although *Pistia stratiotes* is present in this area in ponds and tanks, larvae of Mansonioides spp. were not present on any of these plants examined.

Chandranagar (Hooghly District)—Since a number of filarial patients come from this place to attend the clinic at the school for treatment from time to time, and as on one occasion *W. malayi* infection was detected in one of the patients recently, a survey for filarial infection in this area has just been started. Pistia is seen to be common in water collections here and there along the whole length of the journey from Howrah to Chandranagar, and also in the latter area but the incidence of Mansonioides spp. in Chandranagar is very low (1 in 20).

Ratanpur, a small town in Bilaspur Taluk (C.P.) has a total of 170 persons with filarial diseases (Total population 4732). The filarial lesions observed here are entirely elephantiasis of the extremities. There is no case of genital affection, hydrocele or chyluria.

Microfilaria-rate in this area is 21.12 per cent (124 showing the infection out of 587 persons examined). The infections were solely due to *W. malayi*.

A mosquito survey here showed the following species as the most common ones: *Mansonioides annulifera; M. uniformis; Culex fatigans; C. tritaenorrhyncus* and *Anopheles hyrcanus*.

The species distribution of filarial infection.

With a view to determine the species of filarial parasite prevalent in the different endemic areas, collections of thick smears were made from the following areas after a preliminary local survey in each area :—

Muzaffarpur, Darbhanga, Monghyr, Bhagalpur in Bihar, Krishnagar (Nadia district) and Chandranagar (Hooghly District) in Bengal ; Ratanpur (Bilaspur District) in Central Provinces ; and Mangalore, Udipi and Coondapur (South Kanara District).

Except in Ratanpur all the areas showed moderate degree of filarial infection and the infections in them were due solely to *W. bancrofti*. Ratanpur is a *W. malayi* area showing a microfilaria-rate of 21.12 per cent.

Complement-fixation test for filariasis.

Successful results have been obtained with antigen prepared from dog filaria—*Dirofilaria immitis*—for purposes of detection of filarial infection in man. Because of the difficulty of obtaining this antigen under the present conditions, and at the suggestion of Col. Greval, specimens of 'Horse ascaris' were obtained through the help of Lieut. R. W. Davis, American Veterinary Officer, Assam. The fresh material received was preserved in normal saline with 0.25 per cent phenol. The genitalia of the worms were dissected, emulsified and filtered. Antigens thus prepared from the different tissues of this worm were tried for this test in cases of filariasis. All the tests, however, gave negative results.

Studies on the periodicity of microfilariae.

Dr. John Lowe, M.D., Professor of Tropical Medicine, kindly gave a section of the skin of the lobe of ear from a leprosy patient showing microfilariae. The section was collected about 3 p.m. The patient was subsequently examined for embryos ; while they were seen in the peripheral blood collected at night, none could be found in the blood collected during day time.

In view of the interesting findings in the above case, material for study is being collected from suitable cases and this work is in progress.

Transfusion experiments of hydrocele fluids with microfilariae into hydroceles free from embryos were also carried out. The embryos survive for a period of 18 days. The volunteers are under observation.

Treatment of filarial infection.

The following synthetic compound and drugs were tried on patients with filarial infection with a view to finding their filaricidal properties.

'M & B 736' or 4.4 Diamino-dephenyl ethyl isothionate is a phenamidine compound prepared by Dr. A. J. Ewins of Messrs. May & Baker, London. This compound is used in the treatment of Kala-azar infection and is reported to take an intermediate place between 'M & B 744' and 'M & B 800' in its efficacy. This compound did not appear to be lethal to the filarial parasite.

'*Spirodin*' is the hydroiodide compound of 'Spiriochin' which is a heterocyclic base isolated from *Basia latifolia* made by the Alkaloid Research Laboratories, Calcutta. In one case of chyluria it was reported that this drug had brought about a cure. It was, therefore, tried in a series of cases of chyluria and of early elephantiasis but the results were not satisfactory.

'*Enicostema littorale 'Blume'* This plant is pungent and very bitter, anthelmintic ; and is a popular remedy in the south for filariasis. The whole plant powdered is given orally mixed with powdered cardamom and saffron. This was administered to patients in the early stages of filarial infection who are under observation.

Mist. Calotropis Co., made locally by the Indian Herbal Co., with Ext. Calotropis as the main ingredient. This is reputed to be useful in filarial affections and is commonly used by the practitioners of the indigenous system. In vitro experiments the embryos die in a few seconds in contact with this preparation. Patients with active filarial infection and in early stages of the disease are being treated with this preparation. Results will be recorded after the observations are completed.

A local indigenous remedy for filariasis. In the district of South Kanara the leaves of a herb locally called 'Pachutya' were being extensively used as a remedy for filariasis. Specimens of these leaves were obtained and an investigation is in progress to test the efficacy of this plant extract locally applied as a cure for filariasis.

The treatment of allergy in filariasis with filarial allergen is being carried out as a routine measure and is found to give satisfactory results.

32. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras.

Vaccinia virus.

The field experiments initiated in 1940 to ascertain the relative value of chick membrane lymph and routine calf lymph in the prevention of smallpox have been continued.

During the year, 11,880 doses of Membrane Lymph was manufactured. From issues made to field vaccinators, 544 primary vaccination and 2,415 revaccinations were made. In the absence of an epidemic of smallpox in the experimental range, sufficient data are not available for an appraisal yet.

Recently an attempt has been made to ascertain the degree of immunity conferred against vaccinia virus by re-vaccination of children originally vaccinated with chick membrane lymph and calf lymph in the first year of the experiment. The work is in progress and it is expected that by the end of December some data will be available sufficient for a preliminary report on this.

Fowl red cell agglutination with vaccinia virus.

Chick membrane strains of vaccinia virus produced agglutination of red cells of some fowls, but not those of others. Calf dermal strain failed to agglutinate red cells of any of the fowls so far tested. The conditions under which the agglutination occurs is the subject of study.

Typhus.

Attempts made to adapt a mouse strain of typhus to eggs were unsuccessful. Three strains of typhus rickettsia received in dried form were found to be not viable.

Anterior Poliomyelitis.

Attempts made to isolate the virus in brain and spinal cord of fatal cases clinically diagnosed as anterior poliomyelitis were unsuccessful.

Infective Hepatitis.

Serum from cases of infective hepatitis (obtained 5 to 8 days after the onset of jaundice) was inoculated on the chorio-allantoic membrane of the developing chick. Of the four sera tested, two gave passageable lesions on the membrane up to the seventh passage and one up to the fourth passage, while one did not produce any reaction. Similar results were obtained last year.

Squirrels were instilled intranasally with and given orally the pooled throat washings of three cases failed to react during the period of observation of six weeks.

33. Kala azar enquiry under the Director, Pasteur Institute, Shillong.

During April the enquiry suffered a serious loss by death, while on leave, of Mr. C. S. Swaminath, the Parasitologist of the enquiry and the Officer in charge of the field station at Gauhati and a distinguished research worker. It was later decided to close the enquiry.

The following is a summary of the work done.

I. Experiments to infect white mice with flies fed on kala-azar cases and maintained on blood and raisins.

Seven experiments were performed. In three of these, sandflies which were maintained exclusively on blood feeds after their initial infective feed on a kala-azar case were fed on a mouse, and in four others, the same procedure was gone through except that the infective sandflies were maintained on raisins instead of blood.

In these seven animals, infection took place only in one of the mice in the first series. The animal died 64 days after the first infective feed and was found to have L-D bodies in the spleen.

II. Bionomics of the sandfly.

(1) A comparison of the longevity of sandflies maintained respectively on blood feeds on mice and on raisins respectively, in different seasons of the year, was made. The following are the results :

(1) *Blood fed :*			Maximum life
27/1/44	to	10/2/44	14 days.
6/2/44	to	17/2/44	11 ,,
21/2/44	to	4/3/44	12 ,,
3/3/44	to	15/3/44	12 ,,
18/4/44	to	26/4/44	9 ,,
15/5/44	to	23/5/44	9 ,,
10/6/44	to	21/6/44	12 ,,

(2) *Raisin fed :*			Maximum life
10/1/44	to	23/1/44	13 days
2/2/44	to	15/2/44	13 ,,
13/2/44	to	24/2/44	11 ,,
7/3/44	to	17/3/44	10 ,,
18/4/44	to	27/4/44	10 ,,
10/5/44	to	21/5/44	12 ,,
1/6/44	to	12/6/44	12 ,,

In both the series, over 50 % of the initial number were dead by the 6th day. Thereafter the advantage was in favour of the raisin fed sandflies generally, but it is possible that the greater death rate among blood fed sandflies might have been due to their constant handling for feeding them on mice. There is some evidence that during the period January to June, the months least favouring longevity are April and May.

(b) From collections of wild flies in different months of the year, it would appear that flies are never totally absent, but catches were lowest in January and February. Flies were bred in pots kept at room temperature and the incubator at 28°C respectively, and the time taken by each stage of the life cycle observed.

These observations show that there is no hibernation in the strict sense but with the lowering of temperature, the egg and larval stages are prolonged to double the time taken in the incubator, thus explaining the small catches of adults in the cold months. Growth of larvae during these months is slow. With the advent of warm weather in March, the development of the larvae is hastened with a corresponding increase in the prevalence of sandflies.

(c) Laboratory experiments show that even at 100 % atmospheric humidity eggs fail to hatch. Soil humidity, giving the ova a surface film of moisture, is essential for their hatching. The larvae tolerate excessive moisture, but without a film of water die within a few hours, particularly the early stage larvae.

(d) Further determinations of the pH of the alimentary tract of wild flies were carried out by the capillator method, using two different indicators. The range obtained was between 7.2 and 7.6.

III. Experiments with monkey plasmodium (P. cynomolgi).

Preliminary experiments were performed to transmit *P. cynomolgi* to local monkeys by laboratory bred *A. annularis* fed on an infected monkey, kindly despatched by the Director, Malaria Institute of India. Unfortunately little headway could be made as the local monkeys were apparently resistent to the infection ; they got a transient infection and attempts to infect others by inoculation of blood from these failed.

IV. Miscellaneous.

1. 24,141 sandflies were bred in the laboratory during the period, but owing to a scarcity of suitable kala-azar cases, the greater number of them could not be utilised for infection experiments.

2. Fortyfour cases of suspected kala-azar were encountered at Gauhati and the surrounding villages. Of these 10 were positive for L-D bodies. 19 had malaria parasites only in their blood smears and 15 were negative for either.

3. Over 26,000 sandflies were caught in Gauhati and the surrounding villages, and spraying experiments which were started in selected localities could not be concluded because of various unfavourable circumstances.

34. Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

During this year so far 19 strains of Cl. welchii (13 isolated from cases of gas-gangrene and 6 isolated from samples of human and guineapig stools) were studied in detail. After obtaining them in fresh cultures their pathogenicity was tested by injecting them intramuscularly in white mice. The strains were grown in selective media for toxin production. Fresh filtrates obtained were tested for toxicity as follows.

I. Lethal effect.

The lethal effect of the filtrates was tested by injecting them intravenously in a group of white mice in varying doses. It was noted, that only two filtrates, as also the filtrate of the control strain Cl. welchii S.R. 12, showed lethal action on test animals. The remaining filtrates failed to kill the injected animals.

II. Lecithinase activity.

Recent studies have shown that α toxin of Cl. welchii is closely associated with an enzyme lecithinase. The presence of this enzyme is tested by egg-yolk-reaction. Egg-yolk-saline emulsion was prepared by the method recommended by Van Heyningen. Equal quantities of filtrates and egg-yolk-saline emulsion were mixed and incubated at 38°C. From the time taken to develop turbidity and the degree of turbidity developed, it became possible to group the strains of Cl-welchii into 3 classes, namely, those showing marked, moderate, or weak lecithinase activity as follows :—

I.	Strains showing 'marked' lecithinase activity	.. 2
II.	Strains showing 'moderate' lecithinase activity	.. 13
III.	Strains showing 'weak' lecithinase activity	.. 4

The filtrate of control strain S.R. 12 showed marked lecithinase activity. The filtrates which showed marked lecithinase activity also proved to be lethal to test animals.

III. Haemolytic activity.

Haemolysis brought about by filtrate of Cl. welchii is complicated by the presence of at least two different factors namely α and ϕ. Haemolytic activity of the filtrates was studied by mixing equal quantities of the filtrate and 6% suspension of washed fresh red cells of sheep. The mixture was then incubated at 38°C. Filtrates of all the strains studied in this manner showed evidence of a haemolytic activity which may be due to α and/or ϕ factors. The filtrates also showed evidence of marked haemolysis produced by the ϕ factor alone.

V. Hyaluronidase content.

In view of the fact that, filtrates from only two strains showed lethal and marked lecithinase activity, even though, the cultures of all the strains were pathogenic, it was decided to test these filtrates for the presence of spreading factor namely hyaluronidase. This was done by mucin-clot prevention test which depends on the power of hyaluronidase to destroy the capacity of the muco-poly-saccharide to form a typical mucin clot on addition of acetic acid. The test proper was done as recommended by McClean and Roger. Filtrates from 16 strains showed an unequivocal evidence of the presence of hyaluronidase. In the case of remaining three the results were doubtful. Filtrate of control strain (S.R. 12) also contained this enzyme.

V. Serological reactions of strains of Cl. welchii.

Rabbits are being immunised against 'O' & 'L' types of antigens of Cl. welchii S.R. 12, with a view to produce high titre aggultinating sera for further serological work on strains of Cl. welchii. Sera of these rabbits were tested for agglutination reaction to get an idea as regards the reactivity of these animals. It was found that they were reacting. In order to obtain high titre sera the injections are being continued.

35. Enquiry into methods for the manufacture of solid blood plasma and other problems connected with it under the Director, All-India Institute of Hygiene and Public Health, Calcutta.

Processing of liquid serum.

Pooling.—The International Equipment Co.'s serum centrifuge which arrived early this year was fitted up and the bottles of clotted blood are now centrifuged just as soon as they are received, and then stored in the cold room at 4°C as reported last year. By repeated trials it was found that the transfusion bottles used for bleeding break in large numbers when centrifuged at a speed higher than 1000 revolutions per minute. Therefore, in order to avoid loss of blood due to the breakage of bottles, they are centrifuged at 1000 revolutions per minute for 40 minutes. This has improved the yield of pooled serum from 33–35 % to 45–50 %. Thus the yield of filtered serum will be well above 40 % allowing for the loss of serum in pilot filter, which is between 1 to 2 litres depending upon the number of clarifying and sterilising pads used for filtration.

Filtration.—In essential details the technique followed during the period under review was the same as published in the Indian Medical Gazette (1943, Vol. 78, page 600). 77 batches with a total of 2,410.8 litres of serum were filtered. Of this amount 866.0 litres or 1732 bottles were stocked as liquid serum and 1544.8 litres or 3862 bottles were set aside for drying. Because of the constant vigilance exercised over the technical details of the filtration the contamination rate was kept quite low, but two consecutive batches of serum comprising of 81 litres had to be discarded because of general contamination. Since each and every bottle of the above serum became opalescent after a storage of 3 or 4 days at 80°F, it was obvious that the contamination was due to faulty seitz pads. The laboratory sterility report on one of the above batches indicated that the batch was sterile, thus showing that the contamination was so slight

that it could not be detected in the small amount of serum that was taken for the test. This confirmed the opinion formed from experience gained during the last two years that the best sterility test for the filtered serum is storage at 80°F for 3 weeks and then the visual examination of each bottle turned upside down against a light. If the serum looks transparent one can be reasonably certain that it is sterile.

During storage at 80°F for 3 weeks 16 bottles (or 0.9 % of the total number of bottles of liquid serum) showed contamination. Of this 10 bottles were found to have cracks at the bottom, so only 6 intact bottles were contaminated. Loss due to accidental breakage was only 4 bottles during the period reported here.

Five hundred bottles of liquid serum while being stored at 4°C in cold room showed excessive amount of precipitate of calcium soap and so they had to be refiltered. Besides, this 187 litres of serum had to be refiltered during the year for drying due to shortage of fresh serum.

Processing of dried serum.

As reported last year the ammonia compressor attached to the pilot adtevac plant had broken down. With the cooperation of Messrs Volkart Bros. the compressor has now been repaired although it is not as good as before. The minimum temperature in the brine tank is 0°F as against 20°F obtained previously. Because of these difficulties only one batch of serum could be dried in the pilot plant during this year.

The desivac worked throughout excepting for a breakdown in May last after a run of about 8500 hours. The bearings had got worn out and so the requisite amount of vaccum was not being maintained. Messrs Gresham and Craven fitted up new bearing in place of the old ones and overhauled the whole machine. Since then it has been working without any serious breakdowns. The addition of a low temperature cabinet to the desivac eqnipment has been of immense help in cases of breakdown and also for storing frozen serum bottles.

Cracking of the bottles during shelling in a bath of dry ice and alcohol (-70°C) was a source of great loss but with the cooperation of Messrs Scientific Indian Glass Co. the shape of the bottle, the quality of the glass and methods of annealing were improved, with the result that the breakage is now very much reduced. The total number of bottles cracked was 195 or 5.1% of the total number dried.

The shelling machine was duly modified to enable the bottles to rotate horizontally in the cold bath after a certain amount of serum had frozen in the slanting position. This adjustment gave a more even shell, thereby cutting down the drying time by about 6 hours.

Since the room in which desivac is housed cannot be kept sterile, it was found necessary to fasten caps made of $\frac{1}{4}''$ to $\frac{1}{8}''$ thick cotton sandwiched between two layers of gauze on the mouth of the bottles with the help of rubber bands. This is further covered with a layer of cellophane during shelling. While loading the bottles inside the chamber cellophane is removed. Thus the moisture can easily come out whereas there is almost no chance of any contamination getting in when the vacuum is broken through dry and sterile nitrogen. This is supported by the results of sterility tests of the dried serum.

Although the temperature of serum during drying is a better index of correct operation than the vacuum recorded in the MacLeod Gauge, the makers of desivac have not made any arrangements for taking the temperature records.

Till recently a specially designed glass thermometer made locally, was being satisfactorily used to record the temperature of the serum inside a bottle. Now this has been discarded in favour of the Weston Dial Thermometers received from U.S.A. Because of the cotton cap the temperature of the frozen serum will obviously be higher than what it would be if the mouth of the bottle had been open and there was no obstruction for the moisture to escape. To start with the temperature of serum is -20°C. During loading of the chamber the temperature goes up to about -11°C but by the end of the 2nd or 3rd hour the temperature starts falling and by the 6th hour it reaches -14° to - 16°C where it keeps steady. The pressure is usually about 0.35 to 0.45 m.m. depending to a certain extent on the temperature of the room. The amount of moisture thrown out of the clarifier at this stage is about 200 c.c. per hour. At the end of the 12th hour the circulation of ordinary tap water in winter or water at 15° to 20°C in summer is started inside the chamber. This raises the serum temperature to about -10°C to -11°C where it keeps steady till about the 36th hour ; the pressure remaining the same as before. The amount of water ejected out of the clarifier is about 400 c.c. per hour at this stage. At the end of the 12th hour the heater is switched on and the temperature of circulating water raised to 30°C and at the end of 24th, 36th and 84th hours the temperatures are raised to 35°C, 38°C and 40°C respectively. At the end of 36 hours the temperature of serum again starts falling and may go down to -14°C or -15°C. After the 50th hour the temperature starts going up steadily till it reaches about 33°C at the end of 36th hour and the pressure goes down to 0.15 mm. By the 94th hour the pressure may become 0.05 to 0.075 mm. At the end of the 93rd hour the warm water circulation is stopped and tap water circulation is started, and at the end of the 94th hour the pump is disconnected and the vacuum broken through dry and sterile nitrogen. The bottles are taken out, cotton caps are removed and sterile and dry rubber caps are put instead under a flame in the Processing Laboratory. The rubber caps are coated with a capping solution made out of used cinema films dissolved in ether and alcohol.

During the operation of the drying process every care is taken to see that the temperature of serum does not go above -10°C. If any such tendency is observed, the water circulation is immediately cut off.

The total number of batches dried in the interval reported here was 61, comprising 3598 bottles of 1439.2 litres of serum. The loss due to thawing was 44 bottles or 1.2% of the total number dried. A certain number of such bottles could be reconstituted with pyrogen free distilled water, frozen and dried but this was not possible in all cases because of the serum coming in contact with the cotton caps. Only two bottles were lost due to accidental breakage.

The average moisture content was 0.53%. Arrangements have now been made for further desiccation of the bottles over phosphorus pentoxide and it is expected the moisture content of the dried serum will go down to a much lower figure.

Issue of liquid and dried serum.

The number of bottles of liquid and dried sera issued was 4675 and 2037 respectively.

36. Enquiry into the occurrence of ascites in Hyderabad State under Professor S. W. Hardikar at the Osmania Medical College, Hyderabad, Deccan.

The Indian Research Fund Association Enquiry closed on the 5th October 1944. It was found that a proper house to house survey in several villages in different localities would be the only reliable method of obtaining data regarding the relative prevalence of ascites in different areas. It was not possible to spare enough time for this work on account of College and Hospital duties. It is intended to have this done, if possible through the Medical and Public Health Department, Hyderabad-Deccan.

1. The animal experiments reported in the last note were continued. Their results, however, were inconclusive.
2. Observations on hospital cases with regard to plasma proteins, liver function tests etc., were also continued. A further 56 cases were observed to the end of September 1944, bringing the total from the commencement of the enquiry to 343.
3. Reports of the medical departments of different Indian provinces, teaching hospitals, the data from post-mortem examinations in pathology departments of various colleges, and from insurance companies were studied.

Out of a total of 343 cases of ascites studied in the Osmania Hospital during a period of about two years (from July 26, 1942 to October 5, 1944) 37% were associated with cirrhosis of the liver, cardiac insufficiency 15%, renal disease 11%, tuberculosis 10%, and malignant diseases of the abdomen and pelvis 6.4%. In 15% of the cases no definite signs of cardiac, renal, peritonial or Heyatic disease was found ; a majority of these, under hospitalization and dietetic measures, either made complete clinical recovery or were discharged much improved. Plasma proteins of these cases were found to be very low with a reversal of albumin/globulin ratio. Renal and hepatic function tests on these cases did not reveal gross insufficiency of these viscera. These cases have been classed as nutritional deficiency—ascites. An attempt has also been made to follow-up their progress after discharge from the hospital and the results showed that out of 32 discharged as recovered, 6 are known to be well without recurrence of ascites, a year after recovery and 8 more for periods varying from 2 to 9 months after leaving the hospital. On the other hand the course of the cases of Cirrhosis of liver was unsatisfactory although some of them were just keeping well under hospitalization and a liberal diet. These also showed hypoproteinaemia, and oliguria as important features. The view that the patients of cirrhosis of the liver with ascites, do not survive long enough to undergo paracentesis more than two or three times is not borne out from the present study on more than 100 cases. Further it is suggested that ascites even in cases of cirrhosis of the liver is not entirely due to mechanical causes of portal obstruction and that malnutrition seems to play an important role in the causation of cirrhosis of the liver and ascites.

37. Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

1. Inhibition of activity of succinic and other dehydrogenases by snake venoms and their constituents.

It has already been reported that snake venom like that of cobra venom can inhibit the oxidation of sodium succinate by the brain cells of pigeons. Since the brain emulsion used in these experiments contains besides succinic dehydrogenase, other dehydrogenases such as those of lactic, malic acids, etc. and since the brain cells also contain a certain amount of lactate, the results obtained by using brain emulsion may not furnish a quantitative measure of the inhibition of oxidation of sodium succinate by cobra venom. A more accurate and quantitative measure of inhibition can, however, be obtained if, instead of brain emulsion, succinic dehydrogenase isolated from suitable materials is used, the other experimental conditions being maintained constant. With this object in view, succinic dehydrogenase has been isolated from ox-heart by a method described by Weil-Malherbe. (J. Biochem. 1937, **31**, 290). A suspension of suitable concentration of dehydrogenase is prepared in phosphate buffer and a fixed volume of this suspension is placed along with sodium succinate and methylene blue in the conical flasks of the Warburg's manometric apparatus and the rate of oxidation is studied at 37.5°C. It has been observed that, using $\frac{M}{10}$ sodium succinate the rate of oxygen absorption per hour in the presence of 1% and 0.5% enzyme concentrations are 432 c.mm and 176 c.mm respectively. If, however, cobra venom to the extent of 1 mg. is added to the systems, mentioned above, the rate of oxidation is found to be 208 c.mm and 114 c.mm respectively i.e. reduced by more than 50%.

2. Isolation of a cardiotoxin from cobra venom and study of its properties.

In a previous report experimental evidence was recorded which showed that cobra venom contains a specific toxin which can stop the movement of the heart and that this toxin can be partially separated from the other constituents by fractional precipitation of a 0.5% solution of venom first with Na_2SO_4 and then with NaCl. This investigation was continued further and a cardio-toxin has been isolated in a much purer form than what has been reported previously. The method adopted consists in dissolving the precipitate obtained by treatment with Na_2SO_4, adjusting the pH of the solution to 2.8 and precipitating the toxin from the solution with NaCl at 17% concentration. The precipitate is again dissolved in normal saline and precipitated at pH 2.8 by adding sodium chloride till the concentration reached 17%. The precipitate is redissolved in normal saline adjusted to pH 4.5 and precipitated with NaCl at 24 % concentration. This latter procedure yields a precipitate which has been found to contain 55% of the total cardiotoxin originally present in the starting material. This purified material still contains a small proportion of the toxin which causes respiratory failure.

3. Study of the mechanism of inhibition of acetylcholine by snake venoms and their constituents.

The recognition of the importance of acetylcholine in the transmission of nerve impulse have led to a considerable amount of work on its synthesis by

tissue cells. It has already been reported that cobra venom can inhibit the synthesis of acetylcholine by the brain cells of pigeons. Since then this active principle has been separated from many of the other constituents such as the choline-esterase, the haemolysin and the proteolytic enzymes present in cobra venom. With this purified active principle the mechanism of inhibition of acetylcholine synthesis by tissue cells is in progress.

4. ISOLATION OF AN INHIBITOR OF GLYCOLYSIS FROM COBRA VENOM AND STUDY OF ITS EFFECT ON TUMOUR TISSUES PRODUCED IN RATS BY THE INJECTION OF CARCINOGENIC HYDROCARBONS.

It has previously been reported that cobra venom can inhibit glycolysis by the tumour cells produced in rats by the injection of carcinogenic hydrocarbons. The active principle i.e. the inhibitor of glycolysis has been concentrated with only 10% loss in a fraction which contains about 12% of the original toxic activity of the venom. The effect of this concentrated material on the glycolysis by artificially produced tumour tissues in rats has been investigated in a few cases only (as the majority of the rats died before they developed tumour). These few results, however, indicate that the concentrated material contains both inhibitor of glycolysis and of respiration.

38. Enquiry on typhus under Dr. S. R. Savoor at the Haffkine Institute, Bombay.

I. XK TYPHUS.

(a) *Strains of XK typhus isolated during the period :—*

In collaboration with the military authorities, three strains of XK typhus were isolated. In addition, two strains isolated elsewhere in the military laboratories, were identified to be XK typhus. The animal used in the isolation and maintenance of the strains was the white mouse. The guinea-pig is unsuitable for work on the XK typhus. All the above strains are being maintained in the Haffkine Institute.

(b) *Preservation of the virus of XK typhus :—*

Many attempts were made to preserve the XK virus by drying it from a frozen state. Peritoneal fluid virus, or suspensions of infected brains of spleens in 10% and 5% milk, 10% horse serum, 5% gum acacia, saline, etc., were frozen in a mixture of methyl acetone and dry ice, and dried while in the frozen condition. It was found that the dried virus had lost all virulence. Experiments are proceeding.

(c) *Chemotherapy of experimental XK typhus in mice :—*

Batches of mice infected intraperitoneally with 10 lethal doses of XK virus were fed with 10 representative compounds which are heterocyclic derivatives related to sulphathiazole and sulphadiazine, and derivatives of diphenyl sulphones. These drugs synthesised in the Haffkine Institute have been shown to possess chemotherapeutic action against experimental pneumococcal and streptococcal infections. None of the drugs tested had any chemotherapeutic action against experimental XK typhus.

(d) *Cultivation of R. Tsutsugamushi :—*

Duck eggs were selected for the culture of rickettsia as they were reported by Dutch workers to be better than hen eggs. Work carried out has shown that yolk-sacs can be successfully infected and that small quantities (0.1 c.c.—0.2 c.c.) of inoculum and long incubation (7 days) at temperature of 36°C are important factors for the growth of the organisms.

II. Work on Typhus in Bombay.

(1) From the first of February, Weil-felix reactions were carried out on samples of sera sent for the Widal test. Out of 1,596 samples of sera examined, 75 gave agglutination reactions in dilutions of 1/250 or over.

(2) An analysis of symptoms from 63 hospital cases, (including 15 cases diagnosed in 1943), has been attempted. In most instances, complete clinical data were not available. With a view to get a complete symptomatology of typhus in Bombay, a case sheet has been prepared and has been circulated to the various hospitals, with a request that in future all the clinical details asked for should be recorded in the sheet.

In the present series of 63 cases, the criterion used for diagnosis is a rise or fall in agglutination reaction against B. Proteus OX19. The analysis has shown the following special features :—

Out of the 63 cases, 47 were among Indians and 16 among Europeans. The average duration of fever among these 63 cases was 14.1 days. Among the Indians it was 13.5 and among the Europeans 15.5. Rash was present in 13 (or 20.6 per cent cases), 10 (or 21.3 per cent) being in the Indians and 3 (or 18.7 per cent) in the Europeans. There was only one death in the series, giving a case mortality of 1.6 per cent.

(3) One strain of typhus, X19 in type, has been isolated from Bombay during the course of the year. The technique adopted was as follows :—Five cubic centimeters of blood were drawn from a patient on the 12th day of pyrexia (W.F. titre on the 10th day 1/250+ ; 1/500 neg. ; on the 12th day 1/1000+ ; 1/2500±) and allowed to clot. The serum was completely pipetted out, and the dry clot was ground, suspended in 5 c. cm. saline and inoculated intraperitoneally into a male guinea-pig (400 gms.) placed on a vitamin-free diet for 8 days. Fever and tunica reaction occurred on the 12th day. Investigations are proceeding.

III. Relation between typhus strains in endemic typhus of U.S.A. and of Bombay.

(a) Six guinea pigs convalescent after infection with Bombay typhus were inoculated with the 'Wilmington' strain of U.S.A. All reacted, 2 with tunica reaction. The fever, however, was mild and lasted for 2–3 days, compared with the pyrexia of 5—6 days, and tunica reactions in all the six controls.

(b) In the converse experiment, six convalescent animals of the American strain were found to be completely immune to the strain of Bombay typhus.

The above findings are interpreted to mean that the typhus strain of Bombay is much less virulent than the American strain. This is in accordance with the clinical observation that typhus in Bombay is a mild disease.

IV. A RAPID BED-SIDE METHOD OF DIAGNOSIS OF TYPHUS FEVER.

Since the outbreak of the present war, attempts are being made to diagnose cases of typhus at the bed-side. Some improvements were made on the methods of preparation of antigen. It has been found that a washed centrifuged deposit of Proteus OX19 organisms killed by alcohol, stained with methylene blue and suspended in 0.5 per cent sodium citrate is a satisfactory antigen, stable for 3 months. The readings obtained by the rapid test were controlled in 250 cases by the standard agglutination reaction. There were no false reactions.

A paper entitled "A Note on Rapid Bed-side Diagnosis of Typhus" is ready for publication.

V. TYPHUS IN KASHMIR.

The typhus unit proceeded to Kashmir to see whether typhus fever was present in that State. A report on the investigations carried out was submitted in May, 1944, to the Public Health Commissioner with the Government of India, New Delhi.

VI. ALLEGED POSITIVE WEIL-FELIX REACTIONS IN PREGNANCY AND CANCER

Owing to a recent report that Weil-Felix reaction becomes positive in pregnancy (in 100 p.c.) and in a considerable number of cases of cancer, sera from 70 cases in various stages of pregnancy, 15 cases of parturition and 61 cases of cancer (diagnosed histologically) were tested. The findings of Gratch have not been confirmed. The readings obtained are tabulated below :

Table showing titres of agglutination with Proteus OX19 (alcohol-killed) in cases of pregnancy and cancer.

Titres.

Sera	Total	Negative		Dilution							
				1/25		1/50		1/100		1/250	
		No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Pregnant cases	70	39	57.1	23	32.8	6	8.5	2	2.8	0	0
Cancer cases	61	46	75.4	10	16.3	4	6.5	1	1.6	0	0

39. Enquiry into vaccine lymph under the Director of Public Health, Bombay.

1. PURIFICATION STUDIES.

The urge for the employment of a bacteria free vaccine virus for vaccination has led workers to the adoption of different methods of obtaining the same. Barring the preparation of tissue culture virus which, while inherently abcteria free, is not considered to confer the same degree of immunity as calf lymph,

these methods devoted to the purification of calf lymph with varying degree of success range from the employment of chemicals to the use of X-rays and sonic vibrations. The main criterion of the efficacy of a purifying agent is that it should reduce the bacterial content as far as possible and always below the standard laid down by the British Therapeutic Substances Act, without affecting the potency of the lymph.

A few preliminary experiments with sulphathiazole particularly in its higher concentrations had suggested a trial of this drug as a purifying agent. Experiments were initiated to assess the potentialities of this drug in varying concentrations relatively to those of two other agents which are also in current use for purifying lymph.

Methods and materials employed : Thus the purifying agents and their concentrations used in the experiments were :

1. Sulphathiazole in concentrations of 0.25%, 1% and 2%.
2. Phenol in a concentration of 0.5%.
3. Chloroform process by the usual method in which chloroform vapour is passed through lymph for a particular period of time. This method is the one employed in this Institute for purifying lymph.

As glycerine is known to be toxic to vaccinia virus particularly at higher temperatures, it was decided to employ a buffer solution as a diluent for the virus suspension ; gum acacia which is considered to be a good absorbent for the virus was also added to the suspension in a strength of 2.5% to impart viscidity.

Pulp from three calves was first thoroughly ground in a mortar and mixed intimately. This was weighed and then divided into equal lots, one lot for each set. Sterile citric acid disodium phosphate buffer solution of pH 8.0 with 2.5% gum acacia was used as the diluent. The different concentrations of sulphathiazole in the buffer solution were prepared by adding the required amounts of a 33% solution of the sodium salt of sulphathiazole. The pulp lots were well ground in the various solutions in a dilution of 1 in 6 in a Chalybus machine and then stored in cold at—10°C. The phenol and control sets were similarly prepared and then stored in cold. It was noticed that these sets which got frozen at such a low temperature required more than a couple of hours' exposure to the room temperature before liquifying for purposes of examination.

Bacteriological tests : These were made at various intervals and the results are given in the table on the next page :—

TABLE I.

Lymph ground in	Result of bacteriological tests after:														
	14 days			1 month			2 months			4 months			6 months		
	Colony count per c.c.	Gas producing anaerobes	Streptococci	Colony Count per c.c.	Gas producing anaerobes	Streptococci	Colony count per c.c.	Gas producing anaerobes	Streptococci	Colony count per c.c.	Gas producing anaerobes	Streptococci	Colony count per c.c.	Gas producing anaerobes	Steptococci
1. Buffer solution pH 8.0 with sulphathiazole 2%	5×106	—	—	80,000	—	—	70,000	—	—	70,000	—	—	12,000	—	—
2. do 1%	60×106	—	—	7×106	—	—	1×106	—	—	1,50,000	—	—	90,000	—	—
3. Buffer solution pH 8 with phenol 0.5%	1,700 24 hours	—	—	900	—	—	500	—	—	—	—	—	500	—	—
4. Chloroform by usual process	900	—	—	300	—	—	—	—	—	—	—	—	—	—	—

It will be observed from the table that the action of sulphathiazole in the above concentrations on the bacterial flora of lymph is very slow indeed, while that of 0.5% phenol is comparatively very much quicker though it cannot be compared with the speed of action of chloroform.

Both on account of the freezing of the sets which required exposure to room temperature at every time of testing, and also the very slow action of sulphathiazole in buffer solution, it was decided to repeat the experiments using 50% glycerine and water as the diluent instead of the buffer solution. The experiments were conducted on the same lines and the purity of the various sets tested at intervals as before. The results are tabulated below :—

TABLE II.

in 6 lymph ground in	Results of bacteriological tests after –											
	14 days			1 month			2 months			4 months		
	Colony count per c.c.	Gas producing anaerobes	Streptococci	Colony count per c.c.	Gas producing anaerobes	Streptococci	Colony count per c.c.	Gas producing anaerobes	Streptococci	Colony count per c.c.	Gas producing anaerobes	Streptococci
50% glycerine and water with sulphathiazole 2%	+++	..	..	+++	..	..	++	..	..	90,000	..	..
do. 1%	+++	..	..	+++	..	..	++	..	..	1,20,000	..	..
do. 0.25%	+++	..	..	+++	..	..	+++	..	..	1,75,000	..	..
50% glycerine and water with phenol 0.5%	++	..	..	8,000	..	..	900	..	..	900	..	..
50% glycerine and water and chloroformed by the usual process.	24 hours 400	..	..	200	..	..	—	..	..	—	..	..

+++ Too many colonies. ++ Many colonies.

The above results indicate a slightly accelerated action in the case of sulphathiazole, while no spectacular difference can be noted in the case of either the phenolized or chloroform sets.

Potency Tests :—The potency of these various sets was tested on calves. The tests were conducted on the lines adopted by Cunningham. The lypmh to be tested was diluted to 1 in 1000 in saline and five horizontal scarifications each exactly 1″ long and one above another, were made on the shaved belly of a calf, one dip of the lancet in the diluted lymph being made for each scarification. Accuracy in the length of the scarifications was obtained by making the scarifications in 1″ squares marked by pencil with the aid of a metal stencil. Two calves were used for each test to guard against vitiation of results due to refractoriness in the calf. The results are summarized below :—

1. Sulphathiazole exhibited no inimical action on the vaccine virus when in a concentration of 2% even after a period of 4 months. The sets with the buffer solution and 50% glycerine and water as diluents respectively showed no difference in this respect.
2. In the case of the phenolized sets the buffer solution set showed a distinct decline in potency while the glycerine set maintained its potency unimpaired. The deleterious action of phenol in the frozen condition has been previously recorded.
3. The chloroformed sets also showed absolutely no loss of potency with the passage of time. An additional test was put up in respect of this process of treatment of lymph. One set was repeatedly chloroformed five times, a portion being taken out after each chloroforming. Thus there were five samples subjected to chloroforming once to 5 times. The potency of 3 of these samples, chloroformed once, twice, and three times respectively as compared with the un-chloroformed sample showed no decline over a period extending to 6 months. The remaining two viz. those chloroformed 4 times and 5 times however showed an appreciable loss in potency.

An appraisal of these various results indicates that while sulphathiazole is not inimical to the potency of the virus, its speed of purification of lymph is not encouraging enough to support its adoption as a purifying agent in preference to other agents that are in vogue. This is understandable in that the action of all sulpha drugs is known to be more bacteriostatic than bactericidal in character. In the preliminary experiments in which fairly quick bactericidal action was shown, the concentrations of the drug were 5%, 10% and 15%. These concentrations however gave an indication of affecting the potency of the virus.

As regards the other two agents while chloroform has the advantage of immediate results which are of inestimable value particularly when abnormally large quantities of lymph are in demand at very short notice, phenol, though slow in purifying action, has the merit of protecting lymph against accidental contamination. An additional point in favour of phenol has been noted by Stevenson and Butler that phenolized virus has greater thermostability than unphenolized virus. A preliminary experiment carried out to confirm this finding has not been quite successful, probably due to some unknown factor which may have crept in. Further work in this connection is in progress.

2. Immunological studies.

To stabilise views regarding the average duration of immunity after successful vaccination with the present day calf lymph, it was proposed to collect as much data as possible with regard to the response of individuals to revaccinations with varying intervals between successful primary vaccination and revaccinations, the response being classified as vaccinia response, vaccinoid response and immune response.

Such data have been collected as and when material was available the following figures pertain to those individuals who were revaccinated and also presented themselves for the necessary observations to be made.

Interval between last vaccination revaccination and present revaccination.	Number showing immune response.	Number showing vaccinoid reaction.	Number showing vaccinia reaction.
Less than 5 years	155 (82.1%)	34 (17.9%)	....
6 to 10 ,,	39 (32.3%)	54 (44.6%)	28 (23.1%)
11 to 20 ,,	25 (46.3%)	16 (29.4%)	13 (24.3%)
above 20 ,,	3 (42.85%)	3 (42.85%)	1 (14.3%)

Regarding vaccination history of the above individuals those from the interval groups of "11 to 20 years" and "above 20 years" cannot be vouched for with any degree of accuracy. The first two groups, however, which comprise of the major portion of the persons give a fair indication of the immune status of individuals after varying intervals after vaccination. However, the smallness of figures does not permit any definite conclusions to be drawn.

Attempts are being made to collect further data on the above lines with particular attention to the groups with an interval of less than 5 years.

Serological tests.—Experiments to investigate to what extent these cutaneous reactions can be correlated with serological observations are in the initial stages. It is hoped to present results in subsequent reports.

40. Enquiry into Naga sore under the Inspector-General of Civil Hospitals, Assam, Shillong.

This enquiry was sanctioned from this financial year but it could not be started till September, 1944. Dr. M. N. Lahiri, an officer of the Junior Research Cadre, I.R.F.A., on transfer from the Haffkine Institute Bombay joined the enquiry on the 15th September, 1944. All the posts have been advertised but so far suitable candidates were not available except for that of the media-maker. This incumbent is now having his training at the Pasteur Institute, Shillong. The necessary equipment is also being collected.

At the very outset Dr. Lahiri paid a visit to Panitola and Dibrugarh to make a preliminary survey with regard to the incidence of the disease and to ascertain the existing facilities with a view to operating a field laboratory. From the data collected there is some indication that the disease is greatly influenced by climatic conditions. Sporadic cases might occur throughout the year but epidemic outbursts are common with the onset of rains reaching the peak in the months of July and August. The disease then disappears with the approach of the winter when the weather is dry. In the majority of cases which were examined either the ulcers had assumed a chronic nature or the healing processes had already set in.

Particular attention is being directed towards the investigation of early cases in which day to day examination will be carried out with a view to studying their bacteriological flora. This will also indicate to a certain extent if all the ulcers met with could really be classified under Naga sore. Attempts will be made to obtain pure lime culture of these micro-organisms. Although various

organisms have been incriminated by various observers, none has been proved definitely as the primary cause of the disease. Efforts will be made to show if any of the organisms isolated can initiate an ulcer. For this purpose suitable experimental animals will be selected to reproduce the lesions and later, if possible, this study will be extended to human volunteers. Furthermore, it is not unlikely that the disease may be due to a virus. Studies in this aspect will also be carried out.

41. Enquiry into the fraction of liver most suitable for the treatment of tropical macrocytic anaemia under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

Work at the School for the last several years has shown that crude preparations of liver are more effective in the tropical macrocytic anaemia than the finer preparations like anahaemin. It has also been shown that in some cases a good diet, rich in protein, improves the blood picture to an appreciable degree even in the absence of haematinics. In view of these, also in view of the good results obtained by Davies *et al* (1943) from proteolysed liver in cases of pernicious anaemia, investigations were undertaken to find out the effect of proteolysed liver in tropical macrocytic anaemia. The following method was employed in the preparation of proteolysed liver:—

Liver is digested with papain and the hydrolysate after concentration under reduced pressure is mixed up with glycerine and alcohol as described for preparation of Extractum Hepatis Liquidum in the British Pharmacopoeia 1932. The final concentration is so adjusted that one ounce of the final preparation represents 50 grams of raw liver. The liquid is passed through Seitz pad and filled in 15 c.c. ampoules. Part of the concentrated liquid before addition of glycerine and alcohol is dried to powder in vacuo. The solid on analysis is found to contain 13.3 per cent total nitrogen, 50.3 per cent of which is due to peptone bodies, 3.6 per cent due to meta proteins. The solid contains negligible amount of primary and secondary proteoses and gives 5.13 per cent vanslyke amino nitrogen, 4.8 per cent mineral matter consisting of 0.44 per cent of iron. The preparation has got a sweet flavour and agreeable taste.

Results. Fifteen cases of tropical macrocytic anaemia have been treated by oral administration of proteolysed liver. The routine treatment is to give 2 ampoules of proteolysed liver which is equivalent to 50 grams of raw liver daily for 10 days in the first instance, and if required, a second or even a third course of treatment is given. Of these 15 cases definite results have been obtained in 10, the remaining 5 cases are still under trial. Of the 10 cases showing definite results, the blood picture reached the normal level in 4 cases, after a course of treatment, in 1, after 2 courses of treatment, in 2, and after 3 courses of treatment in the 4th. All these cases have been discharged as cured.

Definite improvement has been found in 3 other cases which are still under treatment, one after a single course of treatment, another after two courses of treatment, and the third one after three courses of treatment. Appreciable improvement has also been noticed in the remaining two cases.

In one case, however, there was no improvement after the first course of treatment with proteolysed liver. In this case other forms of potent liver extract given parenterally as also a number of transfusions have failed to produce any improvement.

Though trial has been given in a few cases only uptil now, the results obtained, so far, appear to be encouraging.

Along with the work with proteolysed liver, investigations were undertaken to find out the fraction of liver which will be most suitable in the treatment of tropical macrocytic anaemia by parenteral administration. Fractionation of liver extract is being done according to the well established method of Cohn et al. The 70 per cent alcohol fraction of Cohn containing the whole of anti-pernicious anaemia factor is evaporated under reduced pressure and dried; this fraction is termed as W. The fraction G of Cohn is precipitated from the concentrate of 70 per cent alcohol extract with the addition of alcohol sufficient to make the final alcohol strength 95 per cent. This treatment gives two fractions, the precipitate which is fraction G of Cohn which is also called here as fraction G and the solution containing vitamins like riboflavin, pantothenic acid etc. This solution of 95 per cent alcohol is also evaporated under reduced pressure to dryness and is termed as fraction V.

The three fractions are separately dissolved in pyrogen free distilled water, containing 0.5 per cent phenol for preservation. The preparation is so adjusted that each c.c. of the final preparation in each case would represent about 10 gms. of raw liver. The solutions are passed through Seitz pad and filled in 2 c.c. ampoules, which after passing the usual bacteriological tests are ready for use by parenteral administration.

Treatment with the different fraction of liver W. G. and V. prepared in the laboratory has recently been started in a few cases. Though it is too early to give any opinion as to the efficacy of any of these fractions, it is gratifying to note that moderate reticulocytosis has been observed in one case treated with fraction W.

Investigation about the efficacy of the different fractions, W. G. and V., will be continued along with the work with proteolysed liver on suitable cases of tropical macrocytic anaemia but it may take a little time to come to any definite conclusion as suitable cases are not always available for trial.

42. Enquiry on schistosomiasis in the army under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

The enquiry started towards the end of September, 1944.

Snails collected from the suburbs of Calcutta were kept under observation to find out whether they had any natural cercarial infection. Those that did not pass any cercaria were used in the transmission experiments. A few laboratory-bred snails were also used.

Two hundred and sixty-two snails were exposed to the viable eggs and freshly-hatched miracidia of *Schistosoma mansoni* obtained from men suffering from the infection. Forty-six snails died within three weeks. These were dissected and no sporocysts or cercariae were seen in them. The remaining 216 snails have been kept under observation. The genus distribution of the snails is as follows :—

Acrostoma	.. 1
Bithynia	.. 41

Gyraulus	.. 36
Indoplanorbis	.. 53
Lymnoea	.. 11
Melanoides	.. 42
Pila	.. 3
Vivipara	.. 29

Beginning from the twenty-first day onwards, the water containing the individual snails has been examined daily for cercariae. Up to the end of November no cercariae have been seen emerging from any snail.

Observations on 93 snails have been concluded and the results are shown in the following table.

Snail.	Number exposed.	Total number of eggs and miracidia used.	Period of observation (in days).
Bithynia	18	659	26—55
Gyraulus	23	664	25—58
Indoplanorbis	33	1032	25—47
Melanoides	12	588	26—57
Vivipara	7	299	27—36

All these snails are still under observation. It may be mentioned that during the exposure, the miracidia were not attracted to the above snails ; they swam about and around the snails, without any attempt to penetrate the soft tissues of the snails. It was not possible, however, to watch all the snails, but one or two snails of each genus were examined under the microscope for several hours to watch the actual penetration if it occurred. No such penetration was observed. The work is being continued.

REPORTS OF ADVISORY COMMITTEES.

REPORT OF THE CHOLERA ADVISORY COMMITTEE.

The Committee met at 10 a.m. on Monday the 27th November, 1944, at the Imperial Secretariat, New Delhi.

MEMBERS PRESENT.

Lieut.-Colonel H. W. Mulligan (*Chairman*).
Colonel E. Cotter.
Dr. B. B. Dikshit.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. S. R. Pandit.
Lieut.-Colonel S. S. Sokhey.
Major M. L. Ahuja.
Dr. K. Venkatraman. } (*Joint-Secretaries*).

The following also attended:

Dr. M. C. Balfour.
Dr. B. M. Das Gupta.
Dr. P. N. Banerji.
Dr. J. B. Grant.
Dr. A. N. Goyle.
Lieut.-Col. L. A. P. Anderson
Dr. C. L. Pasricha.
Dr. R. Mathew.
Dr. V. N. Patwardhan.
Dr. S. K. Chatterjee.
Major T. D. Ahmad.
Major A. N. Duggal.
Dr. J. A. Ankelsaria.
Lieut.-Col. J. H. Gorman.
Major B. N. Khan.
Dr. R. Adiseshan.
Rai Bahadur Dr. A. C. Banerjea.

The five items on the Agenda were fully discussed, and the findings of the Committee in relation to each are given below:—

1. TO DISCUSS THE WORK CARRIED OUT BY THE CHOLERA TREATMENT ENQUIRY UNDER THE DIRECTOR, SCHOOL OF TROPICAL MEDICINE, CALCUTTA, AND TO CONSIDER THE PROPOSALS MADE FOR THE CONTINUANCE OF THIS ENQUIRY FOR 1945–46.

The Committee noted that cases of cholera treated with Sulphaguanidine under field conditions had responded in a very promising way but considered that the number of cases treated was insufficient to reach a definite conclusion on the efficacy of this drug. A request had been received to test a new compound (phthalylsulphathiazole) and the Committee felt that other compounds would probably be evolved in the future. India possesses unique opportunities for evaluating such drugs under hospital and field conditions, and the Committee therefore recognised the need for setting up a more elaborate organisation for this purpose. The Committee recommended that two treatment units should be established, one based on the Calcutta School of Tropical Medicine and the other on the King Institute, Guindy. The Directors of these two Institutes signified their willingness to undertake enquiries of this kind and each was asked to submit detailed proposals for the necessary staff, materials, etc. The Committee recommended that each of these units should adopt a prearranged standard plan of investigation and that they should maintain close liaison so that the results obtained might subsequently be subjected to the same statistical treatment. The Committee considered that in regard to field trials of any given drug, 500 cases of cholera with 500 controls would be sufficient. The Committee stressed the necessity for the careful selection of the medical personnel to whom this work would be entrusted. It was felt that two units of the kind visualised could satisfactorily undertake the testing of chematherapeutic agents likely to prove of value in the treatment of cholera.

In view of the above proposals the continuance of the Cholera Treatment Enquiry at the Calcutta School of Tropical Medicine, in its present form, was not recommended but it was considered that the existing personnel of this enquiry might suitably be absorbed into the proposed new unit to be formed at Calcutta.

2. TO DISCUSS THE USE OF BACTERIOPHAGE IN THE PROPHYLAXIS AND TREATMENT OF CHOLERA.

The Committee reviewed all available evidence regarding the use of cholera bacteriophage which had accummulated since the publication of the report of a special *ad hoc* Committee appointed by the I.R.F.A. in 1934. Individual members and visitors expressed their personal views very freely and the whole subject was fully discussed in all its aspects. The Committee decided that there was as yet no evidence that bacteriophage is as valuable as the recognised measures in common use for the prevention and treatment of cholera. The Committee therefore recommended that:

(1) Governments should not be advised to undertake the manufacture and distribution of cholera bacteriophage on a large scale in replacement of other measures of cholera control such as preventive inoculation, disinfection of excreta, chlorination of water supplies, etc.

(2) Existing centres engaged in the preparation of cholera bacteriophage should undertake further experimental work.

The Committee recognised the need for basic research on bacteriophage but did not feel in a position to make specific proposals for the conduct of work of this kind at the present time.

3. To discuss a "liquid medium" cholera vaccine recently prepared at the Haffkine Institute, Bombay.

Lt.-Col. Sokhey described in detail a new type of cholera vaccine prepared in a fluid casein hydrolysate medium at the Haffkine Institute, Bombay. Col. Sokhey's findings also included a number of important and interesting technical points in regard to the antigenic potency of cholera strains and methods of evaluating the protective properties of the vaccine. The Committee noted with great interest this work carried out in Bombay which is still in the experimental stage, and which may lead eventually to the production of a simpler and possibly more effective cholera vaccine and to a better method of assessing the protective value of cholera vaccine. The Directors of the Central Research Institute, Kasauli and the King Institute, Guindy, expressed their willingness to cooperate with Col. Sokhey in subjecting this new product to independent investigation when the work at Bombay is sufficiently advanced.

4. To consider a letter from the Chairman, Indian Pharmacopoeial List Committee, regarding the inclusion of cholera vaccine and cholera bacteriophage in the Indian Supplement to the B.P.

The Committee considered the advisability of including cholera vaccine and cholera bacteriophage in the official Indian Supplement to the B. P. and decided that for the present these products should not be included.

5. To discuss the future of cholera research in India.

The future of cholera research in India was discussed in very general terms. The Committee recognised that, at the present time, cholera research was receiving very little attention, and were unanimously of the opinion that there is a great need for further investigation of many and varied problems in connection with this disease. The Committee decided that they were not in a position to make any specific proposals for extensive investigations until individual members had the opportunity of giving this subject mature consideration. It was agreed that each member should prepare a note embodying his views on this subject and that these notes should be forwarded to the Secretary for circulation among members. It might be necessary to convene a meeting of the Committee at a later date to discuss this question in much greater detail.

The Committee considered that there was an immediate need for one central agency to prepare monospecific high titre cholera sera to facilitate accurate identification of strains in various parts of India. The Director, King Institute, Guindy, expressed his willingness to undertake this work but stated that he would require a grant from the I. R. F. A. to cover expenses. He was requested to submit a detailed budget at an early date.

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 10 a.m. on November 24, 1944. It was constituted as follows :—

Brigadier G. Covell. (*Chairman*).
Lt.-Col. H. W. Mulligan.
Brigadier M. K. Afridi.
Dr. B. M. Das Gupta.
Dr. Ananthaswami Rao.
Dr. D. K. Viswanathan.
Dr. A. C. Banerjea.
Major Jaswant Singh. (*Secretary*).

The following were also present :—

Dr. M. C. Balfour.
Major A. N. Duggal.
Major A. K. Adhikari.
Major M. Jafar.
Major B. N. Khan.
Major T. D. Ahmad.
Dr. John Lowe.
Lt.-Col. J. H. Gorman.

Proceedings.

1. The report on malaria enquiries carried out during the current year and proposals for 1945-46 were considered.

Existing enquiries.

1. Malaria Institute of India (Research Section).

Research activities have again been curtailed owing to abnormal conditions brought about by the war. Five courses in malariology have been held during the year of which one was for civil medical officers. In all 127 officers received training. This brings the total number trained since the outbreak of war to 448.

Laboratory research has been restricted mainly to the preparation of antisera for determining the source of mosquito blood meals. 2169 tests have been carried out by this method. The technique of the preparation of JSB water soluble stain has been modified to attain greater simplicity. A number of indigenous drugs were tested on monkey malaria, but none of these was found to have any parasiticidal value. A new ICI synthetic drug, M 3349, was also subjected to tests. While the parasites disappear from the peripheral blood after the 3rd dose of this drug, it in many cases did not prevent relapses of *P. knowlesi* in monkeys. The study of mosquito bionomics has been continued and the routine identification of mosquitoes and other insects of medical importance has been carried out as in former years.

Southern India Branch.

An extensive series of studies on the bionomics of *A. fluviatilis*, both larvae and adults, is in progress. Laboratory colonies of *A. fluviatilis*, *A. stephensi* and *Aedes albopictus* have been maintained. A strain of *P. gallinaceum* is also being maintained.

INSECTICIDE AND MOSQUITO REPELLENT ENQUIRY.

An extensive series of experiments, both in the laboratory and in the field have been carried out on insecticides, such as Indian and foreign emulsions, activators of pyrethrins, individual sparklet sprayers, freon bombs, etc ; repellents, such as pyrethrum cream, DMP cream and DMP liquid repellent have been tested. Experiments have also been conducted on DDT with various diluents both as larvicide and insecticide. A number of samples of cloth, camouflage netting, etc. have been investigated to ascertain their protective value against mosquito bites either when sprayed with repellents or left unsprayed.

The standard type of hand sprayer (MISH) has been modified in certain respects.

PROPOSALS FOR 1945-46.

(a) *Delhi.*

1. Systematic testing of insecticidal sprays, mosquito repellents and larvicides and spraying apparatus under laboratory and field conditions.

2. Testing of the therapeutic effects and toxic action of drugs as evidenced by their effect on animal malaria as occasion demands.

3. Intensive study of bionomics of mosquito colonies maintained in the laboratory.

4. Determination of food preferences of various species of mosquitoes by the precipitin test.

5. Attempts to increase the efficiency of the various antisera employed for precipitin tests.

6. Observations on the bionomics of *A. culicifacies.*

7. Epidemiological studies on malaria conditions in certain villages and coolie camps.

(b) *Coonoor.*

1. Observations on the bionomics of *A. fluviatilis* and other mosquito species of the Nilgiris foothills.

2. Epidemiological studies in certain villages in the Nilgiris.

3. Maintenance and study of *P. gallinaceum* and of mosquito colonies.

(c) *Insecticide and Mosquito Repellent Enquiry.*

1. Systematic testing of insecticidal sprays, repellents, and larvicides under field conditions against various vector species in different parts of India.

2. Testing of spraying apparatus under field conditions.

3. Observations on the bionomics of the vector species of mosquitoes in different parts of India.

2. *Statistical Enquiry under Dr. M. Yacob.*

The obeject of this enquiry is to study the basic data from which forecasts of malaria epidemics in the Punjab have been prepared during the past 50 years and to place this method on a sounder basis. As the staff of the enquiry joined towards the end of July, data from two districts only could be collected.

PROPOSALS FOR 1945-46.

It is proposed to continue the work on the same lines.

3. *Filariasis enquiry under Dr. Sundar Rao.*

A field survey was carried out at Jhargram, Midnapore District, Bengal, but no case of filarial infection was detected. In this district the infection is heavy in towns in the coastal belt, moderate in those situated inland and absent in the elevated region of the north west.

Moderate degrees of infection with *W. bancrofti* were found in 4 areas in Bihar and in Mangalore, Udipi and Coondapur (S. Kanara District) on the west coast of India.

Complement-fixation tests with antigen prepared from horse ascaris for human filariasis proved unsuccessful.

Studies on the periodicity of microfilariae were carried out.

Transfusion experiments of hydrocele fluids with microfilariae into hydroceles not containing them are in progress.

Certain drugs have been tested for their values in the treatment of filariasis but without success.

The routine treatment of filariasis with filarial antigen has been continued.

PROPOSALS FOR 1945-46.

1. Further studies on the morphology of the adult filariae parasite in man in India.

2. Field surveys and collection of material for study (mosquitoes, blood smears, adult worms).

3. Studies in the treatment of filarial infection and diseases.

4. In addition to the above lines of work it is proposed to start a small scheme for the control of filariasis in the village of Damda, Drug District, C.P., where the prevailing infection is *W. malayi*. The mosquito vector is *Mansoniodes annulifera*, which breeds in ponds containing Pistia.

4. *Pharmacological enquiry under Dr. B. B. Dikshit.*

Formaline sulphanilamide, Formaline sulphathiazole and N1-methyl thiazoline were tested on monkeys infected with *P. knowlesi*. All these drugs possessed some antimalarial activity but were less effective than sulphathiazole.

The thiazoline compound was more easily absorbed than the other 2 drugs but was more toxic.

The cellular and humoral factors in monkey malaria were also studied by a special technique developed in the laboratory of the Haffkine Institute.

PROPOSAL FOR NEW ENQUIRY.

Filariasis investigation in Orissa under the Director of Health and Inspector General of Prisons.

It is proposed to determine the distribution and incidence of filarial disease in different parts of Orissa and to make investigations as to the species of filariae present and the mosquito vector.

The Committee made the following recommendations :—

EXISTING ENQUIRIES.

1. (a) *Malaria Institute of India (Research Section).*

(b) *Malaria Institute of India (S. India Branch).*

(c) *Insecticide and Mosquito Repellent Enquiry (under the Director, Malaria Institute of India).*

The Committee approved the proposals put forward but again drew attention to the insufficiency of the existing staff to cope with the volume of work which the Institute is called upon to perform.

2. *Statistical Enquiry into the methods of forecasting epidemic malaria under Dr. M. Yacob.* The Committee recommended the continuance of the enquiry. It was noted that Dr. Yacob expects that this investigation will be completed by the end of the financial year 1945-46.

3. *Filariasis Enquiry under Dr. Sundar Rao.* The Committee recommended the continuance of this enquiry but suggested that more attention might be given to the development of control measures against the insect vectors of this disease.

4. *Pharmacological Enquiry under Dr. B. B. Dikshit.* The Committee noted that this enquiry terminated in October 1944 and recorded its appreciation of the extremely valuable work which has been carried out by Dr. Dikshit during its course.

PROPOSAL FOR NEW ENQUIRY.

Filariasis investigation in Orissa under the Director of Health and Inspector General of Prisons. The Committee agreed as to the necessity for carrying out such an investigation, but did not approve of the scheme as now put forward. It was considered that salaries proposed were insufficient to attract workers of the necessary experience and calibre to carry out an enquiry of this scope and importance. It recommended that the Director of Health be requested to submit fresh proposals including the provision of an experienced research worker with adequate staff.

II. The Director of Public Health, United Provinces drew attention to the necessity for the scrutiny of all irrigation schemes by the Public Health Department of the province concerned before sanction, in order to minimise the risk of an increase in the incidence of malaria following their introduction. The Committee noted that adoption of this procedure had proved extremely beneficial in Mysore State and desired to draw attention to the importance of introducing a similar system throughout India in view of the extensive developments in irrigation now under contemplation.

REPORT OF THE ELEVENTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN NEW DELHI, ON THE 27TH AND 28TH MARCH, 1944.

COMPOSITION OF THE COMMITTEE.

Lt.-Col. E. Cotter, C.I.E., I.M.S., Public Health Commissioner with the Government of India (*Chairman*).

Professor S. P. Agharkar, Professor of Botany and Head of the Botany Department, University College of Science, Calcutta.

Dr. K. P. Basu, Lecturer in Chemistry and in charge of the Biochemical Laboratory, University of Dacca, Dacca.

Khan Bahadur Dr. A. H. Butt, Director of Public Health, Punjab, Lahore.

Sir Theodore Gregory, Economic Adviser to the Government of India.

Dr. B. C. Guha, Ghosh Professor of Applied Chemistry, University College of Science & Technology, Calcutta.

Mr. H. R. Stewart, C.I.E., Agricultural Commissioner with the Government of India.

Dr. V. N. Patwardhan, Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Dr. G. Sankaran, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. D. M. Sen, Assistant Educational Adviser to the Government of India.

Major G. Williamson, O.B.E., Animal Husbandry Commissioner with the Government of India.

Dr. W. R. Aykroyd, C.B.E., Director, Nutrition Research Laboratories, I.R.F.A., Coonoor (*Secretary*).

The following were present by invitation :—

The Hon'ble Mr. Somerset Butler, Deputy Director General, Directorate General of Food, New Delhi.

Dr. M. B. Daver, Nutrition Officer, Medical and Public Health Department, Hyderabad-Deccan.

Dr. K. Mitra, M.B.E., Officer-in-charge, Nutrition Scheme, Public Health Laboratories, Bankipore, Patna.

Dr. Mohd. Musa Khan, Nutrition Officer, Public Health Department, Punjab, Lahore.

Dr. K. C. Sen, Officer-in-charge, Animal Nutrition Section, Imperial Veterinary Research Institute, Izatnagar.

Lt.-Col. A. M. Thomson, R.A.M.C., Assistant Director of Nutrition, Medical Directorate, New Delhi.

NUTRITION IN INDIA.

The following report, dealing with the practical and public health aspects of nutrition, was prepared for the Public Health Committee of the Health Survey and Development Committee.

(1) Introduction.

Nutrition is of basic importance to public health. On the one hand, faulty and insufficient diet causes impairment of health and physical development and leads, directly or indirectly, to numerous forms of disease. On the other hand, good nutrition is necessary to ensure a high degree of general health and vitality and is essential for the building of a strong and vigorous people. These facts may be emphasized by the following quotation from the Report of the United Nations Conference on Food and Agriculture (1943) :—

> " The science of nutrition is largely a product of this century and has advanced rapidly since the last war. The work of scientists in many countries has produced a mass of new knowledge of the greatest practical significance. Its bearing on human affairs can perhaps be summarised in four sentences :—
>
> 1. The kind of diet which man requires for health has been established.
> 2. Investigations in many parts of the world have shown that the diets consumed by the greater part of mankind are nutritionally unsatisfactory.
> 3. Diets which do not conform with the principles of satisfactory nutrition lead to impaired physical development, ill health and untimely death.
> 4. Through diet a new level of health can be attained, enabling mankind to develop inherited capacities to the fullest extent."

For these reasons, nutrition must occupy a central position in the public health movement. But it is far from being merely a problem of public health, and the measures which must be adopted for its solution differ widely from those to be followed in the case of other health questions. It is indeed obvious that the solution depends on an adequate supply of food and its satisfactory distribution, and that these in turn depend not only on agriculture and other forms of food production but on the whole economic structure and organisation of the community.

The welfare of a people is so dependent on good nutrition that governments must assume responsibility for assuring it by all means in their power. It is idle to suppose that any easy and immediate solution for a problem affecting 400 millions of people, for the most part poverty-stricken and illiterate, can easily be found. But at least a beginning can be made by the vigorous development of public health nutrition work and the formulation of a food policy which is based on the science of nutrition. The present report is concerned with both these questions.

(2) Scope of the Report.

In the first place, the extent of under- and mal-nutrition* in India is discussed. The relation between insufficient and improper diet and health

* In the strict sense of the terms, " under nutrition " means insufficient food, while " mal-nutrition " implies the wrong sort of food. It is clear, however, that an under nourished individual will almost invariably be mal-nourished also, since he will not obtain enough of the various food constituents needed for health. On the other hand, an ill-balanced diet may be sufficient in quantity and yet lead to malnutrition because of its qualitative defects. In practice under-nutrition and malnutrition usually co-exist and the term malnutrition is often used with reference to general inadequacy of the diet in both the quantitative and qualitative sense.

and disease is then considered. The problem having thus been defined, ways and means of preventing malnutrition and improving the diet of the population are discussed under two heads. First, there are specific measures for improving nutrition which are largely the responsibility of public health and educational departments. Secondly, there is the broader approach by means of the satisfactory development of agriculture, animal husbandry and fisheries, with simultaneous growth of industry leading to a general rise in the standard of living. In this section of the report emphasis is laid on the planning of food policy in accordance with the principles of satisfactory nutrition. Reference is made to the population problem, synthetic vitamins and vitamin concentrates, processed foods and the storage and distribution of food. Finally it is suggested that immediate effect should be given to certain recommendations which fall under the head of short-term policy. Throughout the report detail is avoided, the object being to provide a sketch of the problem of nutrition in true perspective and in correct relation to the numerous other problems with which it is interlocked.

Active research is a necessary part of any successful attack on the problem of nutrition. All progress is based on research and there is abundant evidence that nutrition research can make valuable contributions to human welfare. In all progressive countries large sums are spent on nutrition research, and it is necessary that in India also such research should receive ample encouragement and support, in order to hasten the solution of short and long term problems of nutrition. In October 1943 the Nutrition Advisory Committee prepared a report on the future development of nutrition research, indicating the special problems which require further investigation in India. A list of subjects recommended for research is given in the Appendix.

(3) THE EXISTENCE AND EXTENT OF UNDER- AND MAL-NUTRITION.

There are various methods of obtaining information on this question of which the most important are the following :—

(a) The study of food production statistics, (b) detailed surveys of the diet of sample groups in the population, (c) study of the relation between income and expenditure, and the cost of foods and adequate diets, and (d) the study of vital statistics and the incidence of deficiency diseases and other manifestations of malnutrition.

(A) *Food production* :—It is generally recognised that existing food production data are inaccurate and limited. The total area of British India is 511 million acres and that of Indian States 459 million acres. Statistics of acreage and production are published annually for British India, i.e., for some 53 per cent. of all India, but the accuracy of these figures, notably in areas of permanent settlement, is open to question. Production is calculated from area by applying two factors, the " standard yield " and the " condition factor ". As regards the first, " The Department of Agriculture in every province maintains a statement of the normal or average per acre of land of average quality to which alterations are made, if necessary, on the basis of crop-cutting experiments. Such experiments are, however, rarely planned scientifically to give a reliable guide. The last year for which the quinquennial average is available is 1936-37, while figures for 1941-42 are in the course of preparation "*. The " condition factor " is essentially a correction factor designed to allow for yearly

* Report of the Food Grains Policy Committee, 1943.

changes in yield due to fluctuations in rainfall, etc. Unless both factors are correctly assessed—as under present conditions they are unlikely to be—large errors may be introduced. This is roughly the position in British India with regard to cereals and pulses. Since the great bulk of typical Indian diets is composed of cereals, which usually provide 80–90 per cent. of total calories, it is particularly important that adequate information should exist about the supply of these basic foods.

In the case of vegetables and fruits similar factors leading to errors and gaps in production figures are operative, but the position is worse since the classification is faulty, fruits and vegetables not being satisfactorily separated in the returns. It may be added that accurate statistics of fruit and vegetable production scarcely exist in any country, largely because of the difficulty of taking into account production in small gardens and allotments. Rough estimates have been made of the production of milk, fish and meat, but there is no means of assessing their degree of accuracy.

No Indian State publishes any production figures, so that for 47 per cent of the country there is a complete gap in the production data. Seventy-nine States, with a total area of 258 million acres, publish figures of crop areas only. i.e., there are area figures for 56 per cent. of state territory.

In the case of one crop, cotton, a check on field production data exists since every cotton pressing factory must furnish weekly returns to government showing the actual quantity of raw-cotton pressed by it during the previous week. It was recently found in one province that figures calculated from the latter source were some 40 per cent. in excess of crop production data collected by the usual method.

Whatever their degree of accuracy or inaccuracy, gross production figures whether for India as a whole, British India or for individual provinces and states do not throw light on inequalities of supply and distribution within the areas concerned. They cannot, for example, be used to assess deficits in urban areas.

In spite, however, of their inaccuracies and limitations, the food production data at present available show in a general way the unsatisfactory nature of the national diet. Thus they show that the great bulk of the area under food crops is under cereals, thereby indicating the preponderance of cereals in the Indian diet. The total annual production of cereals was roughly estimated by the Food Grains Policy Committee as being in the neighbourhood of 50–55 million tons during the years 1939-43. As a result of the "grow more food" campaign, production in 1943-44 may have considerably exceeded this figure. If the figure of one pound of cereal grains *per capita* daily is taken as representing requirements, an annual production of 65–70 million tons is necessary to meet the requirements of a human population of 400 millions. In these estimates no account is taken of the percentage of existing cereal production devoted to the feeding of livestock, or to the quantities of cereals, additional to cereals used as human food, which would be needed to cover *adequately* the nutritional requirements of cattle and other animals. According to the findings of modern nutritional science, a satisfactory diet should include, in addition to cereals, adequate quantities of other foods such as milk, pulses, vegetables, fruits, meat, fish and eggs. Per capita milk consumption has been estimated as about 5 ozs. daily, a quantity much below that considered necessary by nutrition workers. The amount of milk produced varies greatly in different parts

of the country. In the Punjab, for instance, the estimated *per capita* production of milk is 19·7 ozs., while in the Madras Presidency it is 3·6 ozs. Throughout most of India the diet of the poorer classes includes milk in negligible quantities or not at all. The recorded production of pulses will provide less than 3 ozs. *per capita* daily, which is again below the desirable limit. Production statistics for vegetables and fruits, with all allowances made for their inaccuracy, suggest that these foods are grown in insufficient quantities, and further, that less nutritious kinds are often produced in preference to those of higher nutritive value. While satisfactory figures for egg, meat and fish production are not available, all attempted estimates agree in showing that production is grossly inadequate, and it is common knowledge that the intake of these foods by the majority of the population in most parts of the country is small or negligible. The production of sugar, refined and unrefined, is estimated as 5·3 million tons, which amounts to approximately 1.3 ozs. *per capita* daily, a quantity considerably below that available in England and U.S.A. in normal times.

Far-reaching changes in food production are necessary to raise nutritional standards to a satisfactory level. It is the task of nutrition workers to lay down the quantities of cereals, pulses, milk, vegetables, etc., which are needed for this objective to be attained (see pp. 131-132). The point to be emphasised here is that existing food production data, in spite of their limitations, show clearly that total food supply is insufficient in quantity and that the diet of the population as a whole is defective in quality, since the protective foods which are needed to supplement the staple cereal grains are not produced in adequate amounts.

The improvement and development of food production statistics are of vital importance, since accurate statistics are necessary for the planning of all-India and provincial food policies. It is strongly recommended that immediate steps should be taken to remedy the position throughout the country.

(B) *Diet surveys* :—The broad conclusions about the unsatisfactory nature of the Indian diet which can be arrived at by a scrutiny of food production statistics are fully corroborated by the results of diet surveys, made in various parts of the country. Some 100 surveys have already been carried out. In a typical survey a quantitative investigation is made of all the foods consumed by a sample group of 15–30 families in a given area for a period of 10–30 days. Intake of the various foods per adult man or consumption unit is worked out and the composition of the diet in terms of food factors, i.e., protein, mineral salts, vitamins, etc., is calculated. Surveys have sometimes been repeated on the same area at different seasons. It is to be observed that while there is inevitably a considerable margin of error in diet surveys, because of the circumstances in which they are made and for numerous other reasons, they provide the most satisfactory means of obtaining a true picture of dietary habits and information about dietary defects. The Nutrition Advisory Committee has prepared (1944) a " Note on the Statistical Control of Diet Surveys " in which the subject and its difficulties are discussed in detail.

It has been found, in surveys of typical urban and rural groups, that the calorie intake of some 30 per cent. of families is below requirements and that even when the diet is quantitatively adequate it is almost invariably ill-balanced, containing a preponderance of cereals and insufficient ' protective ' foods of higher nutritive value. Intake of milk, pulses, meat, fish, vegetables and fruit is generally insufficient.

In terms of food factors, the most important deficiencies are those of proteins of high biological value, fat, vitamin A and carotene, vitamins of the B group, and calcium. Intake of vitamin C is generally low. There is also a deficiency of vitamin D in the diet which may lead to rachitic diseases when this vitamin is not obtained in sufficient quantities by the action of sunlight on the skin. Evidence from the clinical side regarding the incidence and prevalence of deficiency diseases in general supports these conclusions.

(C) *The cost of foods and satisfactory diets in relation to income* :—A good well-balanced diet costs more than a poor diet. The cost of feeding individuals and families at a satisfactory level of nutrition can readily be calculated. If the former is compared with income levels prevailing among the poorer classes, and the amount of money available for expenditure on food on the part of this section of the population, it is at once apparent that a gulf exists between actual and desirable expenditure on food. In simple terms, the bulk of the population cannot afford to purchase a satisfactory diet. Study of income levels in relation to the cost of diets thus throws light on the extent of malnutrition. In times of food shortage, or when the cost of staple foods is abnormally high, the price index of such foods may provide irrefutable evidence of widespread under- and mal-nutrition among the lower income groups.

(D) *Diet and disease* :—The ill effects of faulty and insufficient diet are manifold. The infant, maternal and general mortality rates in India are high, and the expectation of life is low. Among the causes of ill-health and disease and death are contaminated water supplies, lack of sanitation, bad housing, mosquitoes and flies which carry disease, and malnutrition. There is no doubt that malnutrition occupies a very prominent position in this group of evil and lethal agents.

(a) Emphasis may be laid on the high mortality rates among infants, and young children, and among women in the child-bearing period. The general experience of those concerned with infant welfare work supports the view that much sickness and mortality among infants are due to the faulty feeding of mother and child. The infant mortality rate for India in 1941 was reported as 158 per thousand live births, while in certain urban areas the rate reached 250 per mille. In the same year the infant mortality rate in England was 59 per mille. In India infant deaths amount to some 24 per cent. of all deaths, while in England in 1936 they amounted to only 7 per cent of all deaths.

(b) Perhaps even more significant is the high death rate during the early years of childhood. About half the mortality recorded in India in any given year occurs in children under 10 years, the corresponding figure for England being about 10 per cent. The pre-school child, i.e., the child aged 1-5, probably suffers more severely from malnutrition than any other group in the population, and the mortality in this group is exceptionally high. The percentage of all mortality occurring in the age groups 1–5 and 6–10 is 19·4 and 5·6 per cent. respectively as compared with 2·1 and 1·1 per cent. in England. When breast feeding has ceased, children of the poorer classes cannot be supplied with milk from animals as a substitute and are usually fed on an ill-balanced diet consisting of rice or another cereal, with a few vegetables. Their development is impaired and resistance to such diseases as broncho-pneumonia and dysentery—among the most important causes of death in this group—is reduced.

(c) The female death rate during the reproductive period (15 to 45) is higher than the male, and the excess is largely due to mortality from

child-bearing. The vital statistics of England do not reveal a similar trend. Estimates of maternal mortality in various parts of India vary from 16 to 24 per thousand live births, a very high rate. The anaemias of pregnancy, unquestionably associated with malnutrition, are an important cause of maternal deaths. Further resistance to puerperal sepsis, the principal recorded cause of maternal mortality, is often reduced by co-existent anaemia.

(d) Specific food deficiency diseases—i.e., diseases which are due to insufficiency of some food factor or factors in the diet and which can be prevented or cured by making good the deficiency—are in themselves a serious public health problem. The most important are perhaps beriberi, keratomalacia, rickets and osteomalacia, clinical conditions associated with riboflavin deficiency, and goitre. Mention must also be made of various forms of anaemia, which are associated with blood-destroying diseases such as malaria and hookworm and also with malnutrition. Nutritional oedema is frequently seen among ill-nourished children and adults. In addition to well-recognised deficiency diseases, there are numerous other diseases in the causation of which nutritional factors are concerned. These include epidemic dropsy, peptic ulcer, lathyrism, fluorosis, various forms of cirrhosis of the liver, urinary lithiasis, diabetes, tropical ulcer, eclampsia, sprue and certain kinds of chronic diarrhoea, various eye and skin conditions, etc. Further research is needed to elucidate the part played by faulty diet in the causation of these diseases and of numerous other diseases of unknown etiology which are commonly seen in hospitals and out-patient departments.

(e) The general effect of malnutrition in lowering resistance to microbic and parasitic disease must also be emphasised. Experience gained in times of famine amply demonstrate its importance. Attention is especially drawn to the association between malnutrition and tuberculosis, a disease which is steadily increasing in prevalence. It is generally recognised that resistance to tubercular infection is decreased by diet deficiency.

(f) The modern public health movement is not concerned solely with the prevention of disease. It has the broader aim of creating an environment in which each individual can develop his potentialities fully and completely. This is particularly true as regards nutrition. Malnutrition produces states of ill-health and lowered physical efficiency, short of actual disease, which are perhaps more important, because more widespread, than disease itself. Numerous investigations among school children in India have shown that a large percentage of children are in a poor state of nutrition, with consequent impairment of physical and mental growth. Again, in the adult population the ill-effects of malnutrition are widely evident in the shape of low level of general health and reduced capacity for work. On the other side, the striking improvement in the condition of army recruits which takes place after a few months of abundant and satisfactory feeding is highly significant.

The *positive* aspects of the campaign for improved nutrition must be strongly emphasised. Freedom from disease is one thing, abundant health is another. *The goal to be aimed at is the creation of a healthy and vigorous population.*

(3) *SPECIFIC MEASURES FOR IMPROVING NUTRITION.*

(A) *Nutrition in Public Health Departments* :—The facts presented above clearly show that public health nutrition work is as important as other activities of public health departments, such as the prevention of epidemics, the disposal

of sewage, etc. It follows that health organisations must be extended so as to include work in the field of nutrition among their recognised functions.

(a) *Nutrition in the Central Health Department* :—The Central Public Health Department should include a highly trained nutrition specialist with wide experience of the public health aspects of the subject. This specialist will be on the one hand in touch with experts in other branches of public health in the Central Health Department and on the other with the central nutrition research organisation, provincial nutrition sections, and research workers in the nutritional field. He should advise the Central Government on nutritional policy through the Public Health Commissioner.

(b) *Nutrition sections of provincial and state public health departments* :—The section should be in charge of a nutrition officer of the rank and status of an Assistant Director of Public Health with a suitable staff. The staff required will depend on the size and population of the province, the extent of the problem of malnutrition, etc. The training and qualifications of public health nutrition workers will be considered later (F. Training and Propaganda). The section should include a properly equipped and staffed laboratory. The following are among its important duties and functions :—

(i) Study of the composition of foods of local importance.

(ii) Diet surveys.

(iii) Investigations of the incidence of malnutrition and deficiency disease, and of any public health problems associated with nutrition which may arise.

(iv) Apart from these special lines of research, nutrition research generally may be included among the functions of the nutrition section.

(v) The section must pay special attention to the nutrition of ' vulnerable ' groups, e.g., infants, children, expectant and nursing mothers and students. It should work in association with such branches of public health as maternity and child welfare and school medical inspection. The nutrition of industrial groups is also a question of great importance.

(vi) The section should serve as an information bureau on nutrition for the benefit of other departments and the general public, and be responsible for providing material for education and propaganda. It should advise about diet in public and private institutions.

(vii) Nutrition sections should be in close contact with food departments, educational departments, and agricultural, animal husbandry, fisheries and marketing departments. They should co-ordinate nutrition work sponsored by local bodies and private organisations.

(viii) A suitable duty of the nutrition officer will be to act as Secretary of the Provincial Nutrition Committee, referred to below. He must also maintain contact with nutrition research institutes in order to keep in touch with advancing research.

In the public health departments of large municipalities, nutrition work should be developed along similar lines, though no doubt on a smaller scale.

While special emphasis is placed on the employment of specialised nutrition officers in public health departments and on the creation of nutrition

sections, it is equally necessary that all public health workers should have a sound knowledge of nutrition. To this end their training must include satisfactory instruction on the subject. This is particularly important in the case of health workers at the periphery, e.g., health visitors, health inspectors, etc., who are in direct contact with the people.

(B) *Provincial Nutrition Committees* :—The establishment of Provincial Nutrition Committees has previously been recommended by the Nutrition Advisory Committee (1942). These committees should include experts in nutritional science, agriculture, animal husbandry, fisheries, marketing, food administration, economics, etc., and advise governments on questions which concern the nutrition of the population. While their work should be essentially technical in nature, they may also include purely administrative officers, with the object of securing co-operation in their activities from the administrative side.

(C) *Deficiency diseases* :—The prevention of deficiency diseases is an important responsibility of public health nutrition sections. Deficiency diseases tend to disappear with a rise in the standard of living and a general improvement in diet, but it is not necessary to await such developments before launching the attack.

Specific methods can often be effectively used. For example, beriberi may be prevented by the distribution of pure vitamin B_1, or by popularising the consumption of certain kinds of rice, osteomalacia by the use of vitamin D or by changes in social habits involving greater exposure to sunshine, and goitre by the use of iodised salt. Each deficiency disease presents a specific problem of prevention and the most feasible and effective methods of approach can be discovered only by trial of different methods in various areas and groups. For the development and application of satisfactory preventive measures active public health nutrition sections are essential.

(D) *The nutrition of expectant and nursing mothers and infants* :—These groups are specially vulnerable to the ill effects of malnutrition. If a child is to get a good start in life, its mother must be properly nourished, and it must itself receive a satisfactory diet during infancy and early childhood. Special attention must therefore be given to the needs of mothers and infants. In various Western countries measures are taken to supply additional nutritious food to expectant and nursing mothers of the poorer classes and to provide cow's milk to infants for whom breast milk is not available at all or not available in sufficient quantities. It is the responsibility of public health departments to supervise through maternity and child welfare services the feeding of mothers and infants. The further development of such services, with special reference to nutritional activities, is much to be recommended. The approach to the nutritional problems with which they have to deal, and ways and means of making work on this field more effective, require more careful consideration than they have yet received, and study of these questions will be among the most important activities of public health nutrition sections. In a later section of the report it is pointed out that expectant and nursing mothers and infants should have prior claims in the distribution of insufficient milk supplies.

(E) *Communal feeding* :—(1) *School-feeding.*—The development on a wide scale of school-feeding schemes is strongly recommended. This, as experience in other countries shows, would be of the greatest value in the attack on malnutrition and would give abundant returns for money spent in the shape of an improvement on the health of the rising generation. The following passage

from the Report of the Central Advisory Board of Health on "The Medical Inspection of School Children and the Teaching of Hygiene in Schools (1941)" may be endorsed :

"In many parts of India it is the custom for children to have a meal before they leave for school and have no more food until they return home in the late afternoon. Such children cannot be expected to have the necessary energy to devote themselves to their school tasks. It is essential, both from the point of view of education and of health, that all the children should be given a mid-day meal, whether it is brought by the children from their homes or provided at the school by the authorities. Where food is provided by the authorities, parents who are able to pay should contribute their share to the cost of the scheme and in the case of children whose parents are too poor to pay no charge should be made. There is increasing recognition of the necessity for extending the facilities for primary education and for making it compulsory as far as possible, but it is not sufficiently recognised that much of the value of such education will be lost if the child is not adequately nourished.

The main problem therefore is that of providing a satisfactory school meal at as low a cost as possible. No uniform scheme can be devised to meet the requirements of all. Various methods must be attempted for securing for the children of different economic levels some extra nourishment."

Ideally school-feeding should include the provision of a suitable quantity of whole milk. But in view of the fact that whole milk is scarce and dear in most parts of India recourse may be had to other foods. These include skimmed milk and sprouted pulses. Almost any wholesome food which supplies additional calories to under-nourished children is of value. At the same time, it must be recognised that as far as possible food or meals supplied in school should be such that they help to correct the defects in the home diet. If properly organised on this basis, school feeding may be of genuine value in educating children about nutrition and the importance of a well-balanced diet.

School-feeding schemes should be organised by government and local authorities with the co-operation and advice of nutrition sections in public health departments.

(2) *Institutional feeding* :—The satisfactory feeding of children in institutions is a simpler problem than supplying meals to day-school children, but it is one to which insufficient care and attention are often given by the authorities concerned. All children in boarding schools, orphanages, etc., whether these are under state or private management, should receive in adequate and well-balanced diet. It has already been pointed out that the duties of public health nutrition sections include the giving of advice about feeding in residential institutions, e.g., students' hostels, gaols, reformatories, etc., as well as institutions for children.

(3) *The feeding of employees and labour groups* :—In Europe and America great attention has been given to the diet of industrial workers, particularly during the war. At an Industrial Conference in Great Britain in 1943 it was recommended that the war-time measure of providing a balanced meal to industrial workers should be continued when the war is over. This is a question of importance in India, particularly in view of the fact that industry is now expanding rapidly. The establishment by industrial concerns of canteens supplying good food at low cost is to be recommended. In this connection mention should also be made of labourers on plantations and the secretarial

staffs of large business houses. The need for improving the nutrition of workers must be impressed on all employers of labour. The furthering of such developments will be the combined responsibility of governments and employers, but stress may be laid in obtaining the advice of the health department.

The extension of industrial canteens, etc., is eminently desirable in connection with existing food and rationing policy, and in full conformity with that policy. It facilitates food distribution and helps to satisfy the special food requirements of manual workers.

(4) *Catering and nutrition* :—The provision of cheap well-balanced meals through agencies which supply food to the general population, e.g., eating houses, hotels, etc., is of importance as part of the general campaign for raising levels of nutrition. It would involve the education of those concerned in catering. Municipal health authorities, who exercise control over catering establishments of various kinds through licences, should do something to further such developments.

(5) *Community feeding* :—The provision of meals in common to large groups has the advantage of reducing wastage of food and consumption of fuel for cooking, with consequent reduction in cost. If organised on sound dietetic principles, it promotes good nutrition. During recent years the large scale community feeding of urban groups has been developed in Russia. The possibility of similar developments in the future in India, and the advantages referred to above, should be borne in mind. In the case of certain groups, e.g., students in cities, properly organised communal feeding at reasonable cost is definitely to be recommended from the standpoint of nutrition.

(F) *Training and propaganda* :—This subject may be considered under various heads: (a) the education of specialised nutrition workers, (b) the education of those who will be in a position to educate the public or engaged in work in which knowledge of nutrition is of value and (c) the education of the general public.

(a) (i) The question of the education and training of nutrition research workers overlaps with that of the education and training of workers in other fields of medical and scientific research. It will not be considered in this report, which is largely concerned with other aspects of the subject of nutrition. As has already been pointed out, active nutrition research, which implies a body of trained nutrition research workers, is necessary for progress.

(ii) Specialised public health nutrition workers should receive some training in research methods as well as a good grounding in nutritional science, both in the field and the laboratory. They should have at least a year's training in an institution devoted to nutrition research and other branches of the subject. Some knowledge of statistical methods is desirable. They must also have experience of public health work in general, and in particular experience of health work in rural areas, to enable them to deal effectively with nutrition problems in the field.

(iii) In the United States and other countries large hospitals employ *dietitians* whose duty it is to organise and superintend the dietary treatment of patients under the supervision of the medical staff and to ensure that patients receive the best possible diet during their stay in hospital. In India no developments in this direction have as yet taken place, although existing dietary arrangements in hospitals are often unsatisfactory and more could be done to hasten the recovery of patients who are suffering from nutritional diseases.

Moreover, hospitals provide abundant opportunity for nutritional research on a variety of clinical conditions.

All large hospitals should employ a trained dietitian with high qualifications. Apart from highly trained specialists, two possibilities present themselves with regard to the training of workers to supervise dietary arrangements and treatment : (i) special courses on dietetics may be arranged for selected nursing sisters and (ii) graduates of domestic science courses who have taken a course in nutrition may be employed as dietitians after receiving a training in nursing. The Domestic Science course in Madras University now includes a course in nutrition for candidates wishing to specialise in this subject.

In the training of nurses in general, more attention should be given to nutrition. This would help to improve the nutritional treatment of patients in smaller hospitals which cannot employ a full time dietitian.

(b) (i) The medical profession has abundant opportunity of assisting in the attack on malnutrition and furthering the education of the public. At present, in the teaching of medical students, the importance of diet in the causation of disease is not, however, given sufficient prominence. The orientation of medical studies so that greater emphasis is laid on nutrition is necessary. So much disease in India is associated with nutritional factors that there would be every advantage in establishing professorships in nutrition in medical colleges. This would promote research on the prevention and treatment of nutritional diseases and broaden medical education in the desired direction.

(ii) Other professional groups which can play a part in the campaign against malnutrition include administrative officers in general, officers in food departments, school teachers and inspectors of schools, social and economic workers, workers in agriculture and animal husbandry departments, etc. Instruction about nutrition should form part of the normal training of such workers, its amount and kind naturally varying in the different professional groups in question. A necessary preliminary is the education of instructors. It is suggested that special brief courses on nutrition should be given by nutrition research institutes and organisations to professors and teachers of various subjects inter-related with nutrition. Special emphasis may be placed on the instruction of teachers in teachers' training colleges.

(c) All children should be taught simple facts about food and diet, as part of health education. It is the responsibility of central and provincial nutritional organisations to provide the necessary educational material and that of educational departments to impart it to children in suitable form. Attractive booklets, etc., written in English and the important Indian languages, should be prepared. The suggestion is made that, in order to ensure the accuracy of educational material on nutrition, provincial nutrition officers should be co-opted on text book committees. Reference has already been made to the educational value of school feeding.

The importance of the education of the public in general cannot be too strongly stressed. Numerous methods can be followed. These include pamphlets, posters, bulletins, press articles, films, wireless talks, demonstrations, exhibits and museums. Instruction about nutrition should be included in adult educational courses. Here again nutritional and health education in general overlap. Provincial and state nutrition departments have an important part to play in developing educational and propaganda work among the adult population. Public health workers in direct contact with the people, e.g., health visitors, can render valuable assistance in the educational campaign.

Local nutrition committees which include public spirited and influential citizens can further the efforts of provincial, state and municipal health departments to teach the people satisfactory dietary habits and spread knowledge of nutrition.

(G) *Assistance from nutrition research institutes and workers* :—The development of the practical and public health aspects of nutrition must be largely the responsibility of public health nutrition sections. But nutrition research institutes and nutrition research workers in university departments, medical colleges, agricultural and veterinary research institutes, etc., have also an essential part to play. They must supply knowledge, guidance, and advice to ensure that full use is made of scientific discoveries and in general ensure that progress takes place along the right lines.

(H) *The use of milk in the manufacture of non-edible products* :—The nutritional value of milk has been emphasised in previous sections of the report. In view of its importance as an article of diet, it is recommended that the use of milk for the manufacture of non-edible materials should be prohibited.

(4) General Measures.

Much can be done to improve the diet of sections of the population, even under present conditions, by such measures as those referred to in the preceding sections. The general raising of dietary levels throughout the country is a deeper and wider problem involving matters outside the nutritional sphere. Basically it is an economic problem, the solution of which is dependent on the scientific development of agriculture, animal husbandry and fisheries, and simultaneous development of industrial resources. Economic surveys have shown that the poorer sections of the community cannot afford to purchase a nutritionally adequate diet ; they have also shown that with rising income the diet becomes more satisfactory and approaches more closely to approved nutritional standards. An increase in the prosperity of the country, associated with a rise in agricultural production, will thus automatically produce a general improvement in nutrition. It is not, however, sufficient to point out that the latter is dependent on, and will follow, a change for the better in economic conditions. Agricultural and economic policies must be established which have as their primary objective the betterment of diet. A *food policy* is necessary, and this must be firmly based on nutritional science.

(A) *Food policies* :—The United Nations Conference on Food and Agriculture (1943) accepted the principle that governments are responsible for introducing general and specific measures for improving the diet of their people. The Conference, in addition to making recommendations about the feeding of "vulnerable" groups, the necessity for education on nutrition, etc., broadly outlined the method of approach to food policies designed to improve nutritional standards. Its views may be summarised as follows :—

The first step, in planning nutritional problems, is to estimate the average consumption of the various foods by the population concerned, preferably on a *per capita* basis. The rough data about food intake so obtained should be checked by family diet surveys. The state of nutrition of the population should be investigated by medical and public health workers. In this way the defects on the national diet will be made manifest. The adjustment of agricultural and economic policy to correct the defects follows.

On the question of dietary standards, the Conference made the following recommendation :—

"That governments and authorities here represented adopt as the ultimate goal of their food and nutrition policy, dietary standards or allowances based upon scientific assessment of the amount and quality of food, in terms of nutrients* which promote health, and distinguish clearly between these standards and the more immediate consumption goals which necessarily must be based upon the practical possibilities of improving the food supply of their populations."

Because of the inaccuracy of existing food production data, it is impossible to indicate precisely the increase and changes in food production which are necessary to raise the diet of the population to a satisfactory level. Nutrition workers can however lay down standards for requirements of the various important foods which can be used as the basis of all-India or provincial food policies. The problem of drawing up suitable schedules of requirements will be considered by a Sub-committee of the Nutrition Advisory Committee.

The ultimate objective should be the provision of an optimum diet for all, irrespective of income, and plans should be laid to reach the objective by forced march, stage by stage, within a specified period of time. The governmental machinery at the centre and in the provinces necessary to plan and execute food policies cannot be considered in this report. In view of the inequalities in the production and consumption of various foods in different parts of the country, strong central direction is obviously desirable. The distribution of food, with the various problems it involves, is as important as its production.

It is the responsibility of nutrition workers to assess the food requirements of the population on a satisfactory basis. The infinitely more formidable task of fulfilling these requirements lies beyond their sphere of action. It would be outside the scope of this report to discuss the scientific development of agriculture, animal husbandry, fisheries, the mechanisation of farming irrigation schemes, co-operative societies, consolidation of holdings, the need for the large scale manufacture of fertilisers, rural transport, and other questions of equal importance which bear on the problem of increasing food supply and raising dietary levels. Close contact between nutrition workers and those concerned with food production in its various aspects is, however, vitally necessary, if food planning is to be developed along satisfactory lines. Special emphasis may be placed on contact between nutrition and agriculture, animal husbandry and fisheries. Nutritional advice is required in connection with crop-planning, the production of new varieties of seed, and numerous other matters. The activities of central and provincial food administrations, both during the present period of food shortage, price control and rationing and in the future when progressive plans are being developed, should be guided by advice and assistance from nutrition workers. The Nutrition Advisory Committee, the Nutrition Section of the Central Health Organisation and the nutrition sections of Provincial Public Health Departments will all form part of the machinery for securing the necessary contact and co-operation.

Special mention may be made at this point of one highly desirable development. The greater production of vegetables of various kinds in villages would increase the supply of certain vitamins and should be strongly encouraged. Nutrition workers, in collaboration with agricultural departments, can assist in the "grow more vegetables" campaign both by educational methods and by indicating the kinds of vegetables which are nutritionally most desirable.

* Food factors or constituents.

(B) *The population problem* :—The present rate of population increase in India, which shows no sign of slackening, is some 40 million per decade. According to available statistics, the per capita area under cultivation in British India is about 0·86 acre per capita and this figure has not appreciably altered within the last 30 years. With improvement in health services, leading to a reduction in the general, infantile and maternal mortality rates, the rate of population growth will tend to increase rather than to decrease. The view has often been expressed that any potential increase in food supply will necessarily be offset by growth of population, so that no benefit to the individual will accrue and the level of nutrition will remain the same if it does not fall.

There can be no question that the rapid increase in population is of the greatest importance in connection with food policy. It is necessary not only to provide a diet of better quality for the existing population but also to make similar provision for increasing numbers. Nevertheless the rapid growth in population should not be regarded as an insurmountable obstacle, barring all possibility of raising standards of nutrition. The application of scientific methods to food production, the further development of irrigation and land reclamation schemes, etc., should result in increasing output so that growing demands can be satisfactorily met. With simultaneous growth of industry, leading to increased national prosperity, food can be imported in greater quantities from abroad. According to experience in other countries, a rise in the standard of living tends to be accompanied by a slackening in population growth.

The relation between population, food supply and nutrition needs more detailed study than it has yet received. It is disconcerting to nutrition workers to be told that their efforts to improve the diet of the people are futile because of the inexorable increase in numbers. The whole subject should be investigated on scientific lines and expressions of opinion replaced by scientific fact.

(C) *Food Technology* :—(a) *Synthetic vitamins and vitamin concentrates* :—Within recent years there have been remarkable developments in the production of synthetic vitamins. Many of the vitamins required by human beings can now be produced cheaply on a large scale. In some countries, e.g., in England and U.S.A., they have been incorporated in certain of the staple foods of the population. Vitamin deficiency states of various kinds and degrees are common in India and unquestionably large sections of the population would be benefited in health by taking synthetic vitamins. Various deficiency diseases could be prevented by their administration. The use of synthetic vitamins in making good obvious deficiencies in the diet of the vitamins in question is clearly a rational procedure. The administration of vitamins can be combined with that of various minerals, e.g., calcium and iron, when the diet is deficient in such minerals, or (in the case of iron) requirements are increased by blood-destroying diseases.

The development of the manufacture of synthetic vitamins in India is recommended. It must, however, be borne in mind that the administration of synthetic vitamins does not obviate the need for providing sufficient food. For example, if they are given to under-nourished school children, a meal providing additional calories should also be supplied. Their administration as a public health measure should be under carefully controlled conditions. The United Nations Conference on Food and Agriculture remarked that " the *indiscriminate* distribution of synthetic vitamins is not to be recommended as a public health procedure." Here again public health nutrition officers and other nutrition workers have a part to play in organising distribution along satisfactory lines

and observing the results obtained. The cost of synthetic vitamins is obviously a factor of importance.

Shark liver oil is a concentrated source of vitamins A and D and is now being produced on a small scale in India. This promising industry should be vigorously developed. The liver oil itself should be produced in much greater quantities and the industry improved on the technical side so that products of better quality and vitamin A concentrates derived from the oil can be put on the market. Increase in fish liver oil production largely depends on the development of fisheries in general along modern lines, which is of great importance from the standpoint of nutrition. During the war the limited quantities of shark liver oil produced have been in great demand. When peace comes, however, shark liver oil will have to compete with imported fish liver oil and vitamin A concentrates. Measures should be taken to protect the growth of the shark liver oil industry and to develop it along satisfactory lines. Otherwise it may disappear as it did once before in 1870.

Carotene preparations of high vitamin A activity could be used as a source of this vitamin for medicinal and other purposes. Methods of manufacturing such preparation from cheap and abundant vegetable sources should be investigated.

Mention must also be made of the manufacture of dried yeast and yeast extracts, by the growth of yeast in molasses solution. Yeast is of value as a supplement to poor Indian diets, because of its richness in protein and vitamins of the B group. It has considerable therapeutic uses in the treatment of malnutrition and deficiency diseases. Certain strains of yeast can be grown on molasses to produce palatable products of high nutritive value. In the process protein and vitamins are synthesised from a relatively valueless carbohydrate product—molasses—of which a surplus normally exists in India. The possibility of developing the production of "food yeast" at low cost should be fully explored.

(b) *Processed foods* :—The bulk of the population at present lives on foods which are obtained directly from the soil and are prepared for consumption by village and household methods. There are, however, a number of common foods which are "processed" in factories before use. Examples are milled rice, various kinds of wheat flour, vanaspati and refined sugar. In general food manufacturing industries have not developed far in India, though their growth is now being stimulated by the need for producing processed foods for the army.

A well-developed food industry producing a variety of processed foods is desirable from the standpoint of nutrition. It provides a steady market for various food products, facilitates the storage and distribution of perishable foods such as milk and fruits, enables full advantage to be taken of seasonal abundance, thus minimising wastage, and helps to fill gaps in the supply of various kinds of food in areas and seasons in which there is a shortage. The processing of perishable foodstuffs during local and seasonal gluts is in the long run beneficial to consumer and producer alike, since it helps to maintain an even flow and price throughout the year and saves waste. The importance of developing the manufacture of processed milk products, thereby furthering the development of the dairy industry, may be emphasised.

On the other hand, the processing of foods does not increase total food supply. The production of palatable but expensive food preparations will not benefit the bulk of the population. Products which are of no outstanding nutritive value may be popularised by skilful advertising so that those who can

illafford to waste their money on them. Again there may often be loss in nutritive value when the natural product is transformed into the manufactured material.

Administrative control will be necessary to guard against these and other dangers. This includes the fixing of standards for different foodstuffs and an organisation to ensure the observance of legal requirements. The co-operation of trained nutrition workers in the development of food industries is very desirable.

It is recommended (a) that a survey of local and traditional methods of processing and preserving food in India should be made, with reference to the effect of such methods on nutritive value and the possibility of developing any suitable and economical methods on a wider scale, and (b) that at some future date the future of the processed food industry in India, and its repercussions on the problem of nutrition, should be examined by nutrition experts.

(c) *The storage, transport and distribution of food* :—Increased production of food must be accompanied by improvements and developments in its storage, transport and distribution. A few examples of the type of problem falling under these heads may be given. A successful dairy industry involves a satisfactory organisation for distributing milk in good condition to the consumer. The sea-fishing industry needs for its development refrigeration facilities for storage at the coast and transport inland. Improvements in marketing organisations are needed to stimulate the production of various kinds of food.

The great variety of storage, transport and distribution problems which await solution cannot be adequately discussed in this report. They must, however, receive as much attention in the future as other problems of nutrition and food supply, if satisfactory progress is to be made.

(D) *Food standards and food control* :—Food as purchased by the consumer should be uncontaminated and unadulterated and conform to the description given or implied by the vendor. The effective control of food adulteration is an important part of food policy. Satisfactory standards for certain types of foodstuffs, natural and processed, must be laid down and rigorously enforced by legal action.

In the case of certain kinds of manufactured food preparations, notably preparations which are offered for sale with claims that they possess superior nutritive value (e.g., a higher vitamin content), special problems are likely to arise. On this question the following comment was made by the United Nations Conference on Food and Agriculture :

" It is the responsibility of governments to ensure that the consumer is not misled as to the content of foods, vitamin products, food extracts and other preparations derived from foodstuffs. Regulations should be adopted to provide for correct labelling and to ensure that numerical statements are not such as to convey the impression that what are really negligible quantities of a food constituent make a significant contribution to human requirements."

Careful attention must be given to these and similar questions in India. The collaboration of nutrition workers is necessary in framing regulations about food standards and control which can be recommended to government.

(5) Short and long term policy.

The various measures which must be taken to improve the diet of the people have been outlined above. Clearly effect cannot be given to all

recommendations immediately and simultaneously. Some are essentially long-term, calling for sustained effort over a considerable period of years. Others are, however, partially or wholly realisable almost immediately. At the present time, and for several years to come, the country is and will be faced with a difficult food situation. Close attention must therefore be given to practical nutrition problems in the immediate future.

Special emphasis may be placed on the following developments which should take place without delay :

(a) *Contact with Food Departments* :—Steps should be taken immediately to insure the closer participation of nutrition experts in the planning of food policies. It is necessary that full consideration should be given to the nutritional aspects of food rationing and other activities of Central, Provincial and State Food Departments. The effect of administrative measures in the field must be scrutinised and assessed from the nutritional standpoint.

(b) *The establishment of nutrition sections in public health departments* :—It is true that nutrition officers cannot at present be given full training in recognised institutes as envisaged in this report. They can, however, be trained for shorter periods in existing institutions and subsequently employed in public health departments. This will have the effect of making health authorities " nutrition minded ", and enable them to deal more effectively with practical problems arising out of the food situation.

(c) *School-feeding* :—It is difficult to define methods of organising school feeding, or to make concrete suggestions about the type of meal, cost, etc. These questions, being dependent on local supplies and available organisation, must be tackled locally. School feeding is largely the responsibility of provincial and local educational authorities, but it must be encouraged and guided by health authorities. In many parts of the country any type of meal which supplies additional calories to under-nourished children is better than nothing. In rice-eating areas which are short of rice and are partially dependent on other grains, the latter can be used in school feeding. This will help in their popularisation and disposal. As far as possible, however, school meals should be of high nutritive value and designed to correct deficiencies in the home diet.

The *principle* that school-feeding is necessary from the health standpoint must be impressed on Provincial and State authorities, who should then proceed to make their own arrangements, with the guidance of nutrition workers and public health authorities. The local arrangements which are most convenient and suitable can then be made. As experience is gained, satisfactory and efficient methods of feeding should be evolved.

(d) The education of the public, following methods suggested in this report, can be begun immediately.

(e) The problem of improving the accuracy of agricultural statistics demands urgent consideration.

(f) Special emphasis should be placed on an increase in vegetable production, as part of the " grow more food " campaign.

(g) The development of fisheries should receive immediate attention.

(h) As long as the present emergency exists, rationing should be continued. It should be extended to all areas into which it has not yet been introduced. The rationing of various foods other than cereals is to be recommended from the nutrition standpoint. Special measures should be taken to ensure

that expectant and nursing mothers obtain the share of milk supplies necessitated by their special needs.

At the same time the numerous long-term problems referred to in the report must be systematically attacked, as part of broader schemes of reconstruction.

APPENDIX.

Nutrition Research.

A report prepared in October 1943 by the Nutrition Advisory Committee indicates the numerous nutrition problems on which research is required. Subjects recommended by the Committee for research include the following :

(a) A fuller knowledge of the nutritive value of all important varieties of cereals and other foods, including those less widely consumed, is required. Investigations in this field should include the estimation of recently discovered vitamins and trace elements in such foods and the effect of processing and cooking on food values.

(b) Basic research, including studies on the absorption and utilisation of proteins, fats, calcium, iron and other food factors by human beings.

(c) The influence of racial, climatic and dietetic factors on basal metabolism.

(d) Diet surveys in areas not hitherto surveyed and resurveys in some of the areas already investigated.

(e) The socio-economic and psychological background of dietary habits and prejudices.

(f) The continuance of work in order to devise suitable methods of assessing the degree of malnutrition, e.g., anthropometric, clinical and physiological methods. Work on the detection of deficiency disease in its early stages.

(g) The study of numerous diseases which are directly or indirectly associated with faulty diet, by the creation of clinical research units attached to large hospitals, especially in hospitals for children and ophthalmic hospitals.

(h) Research on food technology.

In addition to the above the Nutrition Advisory Committee has recommended closer collaboration between human nutrition research workers on the one hand, and agricultural and veterinary research workers on the other, in the investigation of problems of common interest such as the factors influencing the vitamin content of milk, fluorosis in man and domestic animals, etc.

REPORT OF THE TWELFTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN NEW DELHI, ON THE 24th NOVEMBER, 1944.

COMPOSITION OF THE COMMITTEE.

PRESENT.

Colonel E. Cotter, C.I.E., I.M.S., Public Health Commissioner with the Government of India (*Chairman*).

Professor S. P. Agharkar, Professor of Botany and Head of the Botany Department, University College of Science, Calcutta.

Dr. K. P. Basu, Lecturer in Chemistry and Incharge of the Biochemical Laboratory, University of Dacca, Dacca.

Khan Bahadur Dr. A. H. Butt, Director of Public Health, Punjab, Lahore.

Dr. V. N. Patwardhan, Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Dr. G. Sankaran, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene and Public Health, Calcutta.

Mr. H. R. Stewart, C.I.E., Vice-Chairman, Imperial Council of Agricultural Research, New Delhi.

Major G. Williamson, O.B.E., Animal Husbandry Commissioner with the Government of India.

Dr. W. R. Aykroyd, C.B.E., Director, Nutrition Research Laboratories, I.R.F.A., Coonoor (*Secretary*).

Sir Theodore Gregory, Economic Adviser to the Government of India, Dr. B. C. Guha, Chief Technical Adviser, Government of India, Department of Food and Mr. J. Sargent, C.I.E., Educational Adviser to the Government of India, were unable to be present.

The following were present by invitation :—

Dr. Bashir Ahmad, University Professor of Organic Chemistry, University of the Punjab, Lahore.

Dr. R. E. Dadachanji, Nutrition Officer, Finance Department (Supply), Haffkine Institute, Bombay.

Dr. M. B. Daver, Nutrition Officer, Medical and Public Health Department, Hyderabad-Deccan.

Dr. P. V. George, Nutrition Officer, Public Health Department, Madras.

Mr. W. H. Kirby, Rationing Adviser to the Government of India, New Delhi.

Dr. K. Mitra, M.B.E., Officer-in-Charge, Nutrition Scheme, Public Health Laboratories, Bankipore, Patna.

Dr. Mohd. Musa Khan, Nutrition Officer, Public Health Department, Punjab, Lahore.

Dr. Radhakrishna Rao, Assistant Director, Department of Nutrition and Experimental Pathology, Haffkine Institute, Bombay.

Dr. K. C. Sen, Offg. Director, Imperial Dairy Research Institute, Bangalore.

Lieut.-Colonel A. M. Thomson, R.A.M.C., Assistant Director of Nutrition, Medical Directorate, New Delhi.

1. Nutrition work in Provinces and States.

The Committee considered reports of work carried out by nutrition officers in Bengal, Bihar, Punjab and Hyderabad. A brief account of the work is given below :—

Bengal: The public health organization in Bengal does not include a nutrition officer. The need for such an officer during the famine was acute, and in the circumstances the Government of Bihar was asked to lend the services of their nutrition officer for a period of a few months. The officer in question worked in Bengal from March to June 1944, at a period when the worst of the famine, as far as lack of food and starvation are concerned, was over, but when the people were still suffering from malnutrition, and epidemic disease was rampant.

It was not possible to make detailed dietary surveys to discover the amount and kind of food actually being consumed, since the people were uncooperative. Rough and ready surveys of a small group of 10 families showed a low intake of rice per consumption unit daily, varying from 3 to 11 ozs. This was supplemented by *dal* and to some extent by potatoes.

Clinical surveys revealed that the state of nutrition of considerable sections of the population was still very poor. Groups of children and adults in villages in various districts were examined and some 80 per cent were found to be in a bad state of nutrition. A noticeable feature was the high prevalence of scabies. The causes of this epidemic are not fully understood, and at present the part played in its causation by malnutrition and such factors as insufficiency of clothing, lack of washing facilities, and opportunities for contagion existing under famine conditions cannot be adequately assessed. Vitamin deficiency diseases were uncommon. At the time of the investigation famine oedema was rarely seen among the general population, a fact which can be ascribed to the wide-spread distribution of relief to destitutes and to the removal to famine hospitals of any cases of famine oedema which may have occurred at this period.

An improved scale of feeding in destitute homes and workhouses was drawn up. This was forwarded to Government by the Director of Public Health and accepted in April 1944. The scale came into effect on May 1st. Various defects in the feeding of destitute homes and workhouses were brought to the attention of the Revenue Department.

It was noted that the scarcity of milk in Bengal was likely to have an adverse effect on the health of growing children. The need for increasing milk supplies was strongly emphasised in the report of the nutrition officer.

Bihar: Dietary surveys were made of groups of families surveyed four years ago. It was found that the intake of cereals and pulses was approximately the same as in the earlier investigations. There was, however, a reduction in the intake of vegetables of 25 per cent, and in that of milk products of 50–80 per cent. Investigations of the state of nutrition of children were also carried out. Of 800 children below the age of 14 in the Bihar sub-division, some 26 per cent were found to be in a poor state of nutrition. In the district of Manbhum the percentage of poorly nourished children was 40. No striking increase in the prevalence of food deficiency disease was observed.

Human feeding experiments were carried out with the object of investigating the metabolism of calcium and phosphorus in individuals consuming diets of the kind ordinarily taken by the people of the province. Educational

and propaganda work was carried out as usual. A stall dealing with food and nutrition was organized in the War Services Exhibition held in Patna in January 1944.

Punjab: A survey of diet and nutrition in the Ambala district was begun in October 1944. This investigation is being carried out by a number of officers in the Health Department, with the collaboration of an investigator employed by the Board of Economic Enquiry. It is proposed when necessary to analyse foodstuffs grown in the district for chemical constituents and vitamins. A statistical officer of the Punjab Health Department has been associated with the survey in order to assist in the planning and analysis of the survey work.

A survey of the diet of the boarders in the Khalsa College, Amritsar, was carried out. The diet was found to be satisfactory in every respect, and the boarders were in a good state of nutrition. Advice was given to the Inspector-General of Police, Punjab, about the diet of the Punjab Police Force.

The attention of the Government was drawn to the important question of the formulation of a sound food policy in the Punjab. Stress was laid on improving the state of nutrition of the masses by assuring a satisfactory diet to every individual irrespective of cost. It was concluded that the amount of land required per capita for the production of a balanced diet was 1.15 acres. Careful watch was kept on the food situation in the Punjab during the year under review. There was no complaint of scarcity or shortage of food from any part of the province, urban or rural. A Provincial Food Advisory Board has been set up under the chairmanship of the Food and Development Minister, and the Director of Public Health has been appointed a member of this Board. The nutrition officer has become a member of the Lahore Food Advisory Committee.

The experiment on the prevention of a simple goitre by the use of iodised salt has proceeded satisfactorily. Evidence has been obtained that in the villages in the Kangra Valley chosen for experiment the incidence of goitre has been reduced. The Punjab Government is now considering the possibility of making iodised salt available throughout the Kangra Valley in place of common salt, by means of legislation if necessary.

Educational and propaganda work has been continued. A pamphlet entitled " Hints on the constitution of balanced diets and cheap substitutes for common foodstuffs in the Punjab " has been printed in English and the common Indian languages and given wide publicity throughout the province. A nutrition section was organized in the War Services Exhibition which visited Lahore in April 1944. On the occasion of the annual vegetable show at Lahore in February 1944, a section on nutrition dealing with vegetables and their nutritive value was set up. Radio talks and lectures on nutrition have been given from time to time by the nutrition officer.

Hyderabad: Surveys of diet and nutrition have been carried out in various areas in order to obtain information about the present food situation and its effect on the diet and health of the population. In general, no striking change in diet was observed. In certain areas, however, there was some increase in the incidence of vitamin deficiency disease.

A Nutrition Advisory Committee consisting of 21 members has been set up with the Hon'ble the Revenue Member as its President and the nutrition officer as its Honorary Secretary.

The problem of fluorosis in Hyderabad has been investigated. "Mottled enamel" was found to be very common in certain districts bordering on the Madras Presidency and bony changes in adults were observed. A correlation was observed between the prevalence of mottled enamel and the fluorine content of water supplies. Some evidence was obtained that the incidence and severity of fluorosis are related to economic and nutritional status as well as to the concentration of fluorine in drinking water. On the propaganda side, lectures were given in villages and schools and 15 leaflets written in simple language were printed for free distribution. Two radio talks were given. Assistance was provided by the nutrition officer in organising the nutrition section of the War Services Exhibition which visited Hyderabad in November 1944. This section proved a great success and attracted great interest on the part of the public.

The Nutrition Advisory Committee expressed its appreciation of the work carried out during the year in the provinces and states in question.

2. The budget of a Provincial Nutrition Department.

In its March 1944 Report the Committee recommended that Provincial and State health departments should include a nutrition department in charge of a nutrition officer and outlined the duties and functions of such nutrition departments. No budgetary details were, however, given in the previous report, a fact which was pointed out by the Public Health Committee of the Health Survey and Development Committee when the Nutrition Advisory Committee report was presented to it in July 1944. A suggested budget for a Provincial Nutrition Section is attached to this report (Appendix I).

3. Contact with the Imperial Agricultural Bureau.

The Committee considered the following recommendations made in para 73 of a report on the Imperial Agricultural Bureau by a Committee under the Chairmanship of the Rt. Hon'ble Lord Hankey, G.C.B., G.C.V.O., F.R.S., (1943) regarding contact between nutrition workers in England and various countries in the East including India, and Tropical Africa.

"Para 73. Sir John Orr brought out a difficulty experienced by the Bureau in supplying information for the application of the science of nutrition in tropical countries. The Bureau has no difficulty in covering efficiently all the fundamental scientific literature on nutrition, whether animal or human. It has no difficulty with papers dealing with the application of that scientific knowledge to the problems of nutrition in countries in the temperate zones, for it knows fairly well the general, social and economic background against which those are written. It does find difficulty at present in dealing with applied nutrition in tropical countries. Nutrition is attracting increasing attention in these countries. But the total volume of the literature therefrom, dealing with the application of nutritional science to the feeding of men and animals, is, at present, too small in volume and too disjointed to provide, in itself, that general knowledge of conditions with which those working in the locality are familiar. When work on nutrition does develop on a scale commensurate with the magnitude of the problem this difficulty will lessen. Meanwhile, it would help to solve the difficulty and help the work in those countries, if one junior and one senior assistant were to work for a year as members of the

staff of the Bureau, and so, not only become familiar with the general literature on nutrition, but also with the methods adopted in investigations into diets, etc. They would also provide on their return a link between the Bureau and those engaged in the investigation of problems of immediate local importance. They would thereby increase the usefulness of the bureau as an all Empire centre of information. This proposal would cover the East- India, Burma, Ceylon, Malaya- and Tropical Africa.

We recommend the Executive Council to consult the Government of India, on the desirability of some officers from India working at some fairly early date, for a year in the Bureau of Human and Animal Nutrition."

The Committee endorsed the suggestion put forward by Lord Hankey's Committee and considered that advantage should be taken of the opportunity offered, and that suitable workers from India should be sent to work for a year as members of the staff of the Bureau at the earliest possible opportunity.

4. Presentation of the March 1944 Nutrition Advisory Committee Report on " Nutrition in India ".

The Committee noted that the report of its eleventh meeting held on the 27th and 28th March 1944 was presented to, and accepted by, the Public Health Committee of the Health Survey and Development Committee in July 1944.

5. Review on Basal Metabolism.

In October 1943, the Nutrition Advisory Committee recommended the preparation of review articles on various subjects. Dr. V. N. Patwardhan was asked to prepare a review on " Basal Metabolism ". This review has been completed and was considered by the Committee, which expressed its appreciation and thanks to Dr. Patwardhan and recommended that the review should be published.

6. Report on Nutritional Requirements.

A report by a Sub-Committee on nutritional requirements was considered. The preparation of this report was recommended by the Nutrition Advisory Committee in March 1944. After considerable discussion and certain alterations which were agreed to by the framers of the report it was accepted by the Committee. It has been printed as a separate report and a copy is attached to this report.

7. Nutrition Research Units in Calcutta and Lahore.

At the meeting in December 1943, it was decided that the question of forming nutrition research units under Dr. Guha and Dr. Ahmad in Calcutta and Lahore respectively should be brought up for consideration in course of 1944. Dr. Guha is at present employed in the Food Department and is absent in England on a technical mission. In the circumstances the formation of a nutrition research unit under his direction was not considered. The Committee was also informed that Dr. Ahmad would not be in Lahore for a considerable part of the year 1945 and hence could not at this stage undertake the formation of a nutrition research unit. The matter was, therefore, shelved for the time being.

8. Diet Surveys.

The Committee discussed the adequacy of the information accumulated by diet surveys for the proper planning of food production. The question of diet surveys has previously been before the Committee on several occasions. In 1943 it considered a detailed memorandum on " The Statistical Control of Diet Surveys " which was printed in 1944 after certain emendations suggested by the Committee had been made. This memorandum stresses the need for further surveys, particularly in parts of the country in which no surveys, or an insufficient number of surveys, have been made and there is not enough information about dietary habits. The same point is made in the Report of the Sub-Committee on " Nutritional Requirements ", attached to this report.

In the course of discussion various points emerged. It was pointed out that while the results of surveys carried out before the present food situation developed throw light on dietary habits and hence provide some guidance for food planning, little or no knowledge is available about the effect of the present food situation on the diet of the people. The effect of rationing on diet, for example, has not been investigated. Doubts were expressed whether at the present time it would be possible to obtain the cooperation of the people and obtain accurate data about food intake. The most suitable organizations for carrying out diet surveys are provincial and state nutrition departments; according to the recommendation of the March 1944 report, this is one of the important activities of such departments. On the other hand, the existing personnel of nutrition departments is insufficient to enable diet survey work to be increased to any great extent.

Both Food and Agricultural Departments must have more information about dietary habits in order to plan their future programmes. Difficulties include the lack of suitable personnel at the present time and the failure of a number of the Provincial Governments to appoint nutrition officers with requisite staff.

Survey workers must be trained before starting investigations in the field. It was pointed out that workers engaged in the actual collection of data about food intake need not necessarily be medically qualified, but that it was desirable that surveys should be coordinated and assessed by a medical worker who can deal with the problems of the medical evaluation of results and the state of nutrition of the population surveyed.

It was recommended that the question of devising ways and means of carrying out further surveys without further delay should now be taken up by the Department of Education, Health and Lands in conjunction with Provincial Governments. The " Note on the Results of Diet Surveys " prepared by Dr. Aykroyd in 1939 should be brought up to date by the inclusion of the results of surveys made since that date.

9. Soya-bean.

The Committee considered and accepted the report of the Sub-Committee on Soya-bean which is attached to this report.

10. The inclusion of certain therapeutic substances in the official Indian Supplement to the British Pharmacopoea.

The Committee considered a reference from the Chairman of the Indian Pharmacopoeial List Committee enquiring as to the advisability of including

preparations of yeast like marmite, amla powder, shark liver oil and red palm oil in the supplement and also to suggest standards for any preparations whose adoption was recommended. It felt, however, that this matter could not be dealt with without careful and prolonged consideration. The proposal that members of the Committee should be asked to comment by correspondence on the substances in question was accepted.

11. Existing and proposed enquiries.

The Committee recommended the continuation of the following enquiries :-

(i) Nutrition researches under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, Coonoor.

(ii) Ascorbic acid enquiry under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta. It was recommended that in order to take stock of the results obtained in this enquiry a report should be prepared for the next meeting of the Committee on the work carried out since the start of the enquiry.

(iii) Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay. A report should be prepared for the next meeting of the Committee summarising the results obtained since the start of the enquiry.

(iv) Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.

(v) Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipore, Patna.

(vi) Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

(vii) Nutrition research unit under Dr. K. P. Basu at the Dacca University, Dacca.

(viii) Nutrition research unit under Professor S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(ix) Enquiry into methods of treating sick starving destitutes in Calcutta based on the All-India Institute of Hygiene & Public Health, Calcutta.

(x) Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.

(xi) Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

(xii) Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

(xiii) Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras.

The following enquiries are terminating this year :—

(i) Enquiry on the role of manganese on the biological synthesis of ascorbic acid under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

(ii) Enquiry on the deterioration of vitamin A in liver oils, concentrates and other preparations under Mr. B. N. Banerjee at the Indian Institute of Science, Bangalore.

(iii) Enquiry into (1) tests of the biological value of soya-bean protein (2) growth tests with soya-bean, (3) human experiments with soya-bean under Drs. W. R. Aykroyd, K. P. Basu, Bashir Ahmad and V. N. Patwardhan. (See the report of the Soya-bean Sub-Committee).

The following new enquiry was recommended :--

Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary protein to blood and muscle haemoglobin under Professor M. Damodaran, Director, University Biochemical Laboratories, Triplicane, Madras.

It was recommended that the officer in charge of this enquiry should consult the Pharmacological Department of the Medical College, Madras, about the proposed scheme.

The following enquiries were not recommended :—

(i) Enquiry on epidemic dropsy under Dr. S. N. Sarkar, Lecturer in Biochemistry at the University of Dacca, Dacca.

(ii) Somatometric survey in the United Provinces under Professor A. M. Khan, Head of the Department of Anatomy, University of Lucknow, Lucknow.

(iii) Enquiry on vitamins B and C under Professor N. M. Basu, Professor of Physiology at the Presidency College, Calcutta.

(iv) Enquiry on the vitamin A content of the principal foods of the Punjab and the preparation of vitamin A concentrate by distillation of fish liver oil under very low pressure under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.

An application was considered for an enquiry on the effects of prenatal diet on the clinical course of pregnancy, puerperium as well as on the condition of the infant at birth under Mr. N. C. Datta, Assistant Professor of Biochemistry at the Grant Medical College, Bombay. The Committee did not consider that this scheme could be supported in its present shape. It was decided to await a report by Dr. S. Pandit, W.M.S., Director, Maternity and Child Welfare Bureau, New Delhi, who is expected to visit Bombay shortly. The report when received will be circulated to the Committee by the Chairman.

APPENDIX I.

The following was suggested as the budget of a provincial nutrition section. As pointed out by the Nutrition Advisory Committee in its March 1944 report, the staff required will depend on the size and population of the province, the extent of problems of nutrition, etc. In the case of laboratory work there are already in certain provinces nutrition research units financed by the I.R.F.A. Where such units exist the responsibilities of the public health nutrition section as regards laboratory research will be reduced, since the former can undertake the investigation of various problems.

Capital expenditure (equipment)

Nutrition laboratory	Rs.	75,000
Animal cages etc.	„	5,000
	Rs.	80,000

Recurring expenditure (annual)

Chemicals etc.	Rs.	2,500
Food etc.	„	1,500
Travelling allowances	„	7,000
Contingencies	„	5,000
Average annual cost	Rs.	16,000

Staff.

1 Chief Nutrition Officer (Pay as an Asst. Director of Public Health say Rs. 900 p.m.)	Rs.	10,800
3 Field workers—Rs. 300—20—500	„	10,800
2 Peons—Rs. 20—2—30	„	600
	Rs.	22,200

Laboratory.

1 Chief Chemist (Chemistry and Biochemistry)—Rs. 300—20—500	Rs.	4,800
1 Chemist—Rs. 150—10—250	„	2,400
3 Laboratory assistants—Rs. 50—5—100	„	3,600
2 Animal attendants—Rs. 20—2—30	„	600
2 Peons—Rs. 20—2—30	„	600
	Rs.	12,000

Office.

1 Statistician—Rs. 150—10—250	Rs.	2,400
1 Stenographer—Rs. 75—5—150	„	1,200
2 Typist clerks—Rs. 60—5—100	„	1,920
1 Artist—Rs. 100—5—150	„	1,460
2 Office peons—Rs. 20—2—30	„	480
	Rs.	7,460

Summary.

Capital expenditure on equipment	Rs.	80,000
Recurring expenditure on chemicals and miscellaneous	„	16,000
Recurring expenditure on staff	„	41,660
	Rs.	1,37,660

NUTRITION ADVISORY COMMITTEE, INDIAN RESEARCH FUND ASSOCIATION.

REPORT OF THE SUB-COMMITTEE ON NUTRITIONAL REQUIREMENTS NOVEMBER 23rd, 1944.

I. Introduction.

The present food situation in India has revealed the necessity of planning in matters of food production, procurement and distribution. During the early years of the war there was no agency or organization in possession of accurate information about the food position as a whole which could forestall or meet any critical situation. The "Grow More Food" campaigns sponsored by central, provincial and state governments were a step in the right direction, but such measures were not in themselves sufficient to make good the food shortage caused by failure of imports, war demands, transport difficulties, drought, cyclones, floods, crop disease, etc. The devastating famine in Bengal and the serious food shortage in other parts of the country have provided a warning that India's food position is highly precarious and that most energetic steps are necessary to meet the demands of the present situation and to raise the general standard of nutrition of the population.

The Food Department of the Government of India was established in December 1942. Its first task was to deal with urgent problems of food procurement and distribution. Provincial and state food departments have also been created to deal with the same problems. There is a feeling in the minds of the public that food departments are a war-time measure to be scrapped when normal conditions return. But actually the need for such departments will by no means disappear when the war is over; on the contrary they will be needed to deal with short and long-term food policies and must play a prominent part in post war reconstruction. We cannot envisage with equanimity a return to the era of uncontrolled production and distribution, with the buying and selling of foodstuffs controlled entirely by individuals actuated by the profit motive. The question of food supplies is too important to be left to private individuals. The only agency competent to deal with the problem as a whole is a properly constituted and functioning department of the Central Government working in collaboration with provincial and state departments. A quotation from the Report of the Eleventh Meeting (March 1943) of the Nutrition Advisory Committee of the Indian Research Fund Association explains clearly the point of view held by the nutrition workers in the country.

"The welfare of the people is so dependent on good nutrition that Government must assume responsibility for assuring it by all means in their power. It is idle to suppose that any easy and immediate solution for a problem affecting 400 millions of people for the most part poverty-stricken and illiterate can easily be found. But at least a beginning can be made by the vigorous development of public health nutrition work *and the formulation of a food policy which is based on the science of nutrition*" (The italics are ours).

Hitherto food policy has been concerned mainly with the supply of the minimum quantities of cereal grains needed to sustain the population at a low level of nutrition. It is necessary that consideration should be given to *all* the foods which are needed to make up a well-balanced and satisfactory diet which is essential for maintenance of good health. We propose in this report to provide a

basis for the formulation of satisfactory food policies. We are concerned here not with the present difficult food situation. Our purpose is to establish objectives for long-term planning by assessing the quantities of different foods required to balance the diet and indicating the changes in existing production necessary to attain this end.

II. Nutritional requirements.

The formulation of human nutritional requirements in terms of essential nutrients is by no means a simple task. Knowledge about requirements of a few nutrients is still incomplete and existing estimates may require modification in the light of future research. But in general it is already sufficient for practical purposes. The following table of requirements is based mainly on (a) the recommendations of the Technical Commission on Nutrition of the League of Nations Health Organization, (b) those of the Food and Nutrition Board of the National Research Council, U.S.A., and (c) the experience of various workers in the field of nutrition in India.

TABLE I—DAILY REQUIREMENTS OF CALORIES AND SOME ESSENTIAL NUTRIENTS.

		Net calories.	Pro-teins.	Fats.	Ca. (Cal-cium).	Fe. (Iron).	Vit. A I.U.	Thia-min (Vit. B_1).	Vit. B_2 com-plex.	Ascorbic acid.	Vit. D. I.U.
			g.		g.	mg.		mg.		mg.	
MAN (55 Kg. or 120 lbs.)	Light or sedentary work.	2400	82								
	Moderate work	3000	82								
	Very hard work	3600	82		1·0						
WOMAN (45 Kg. or 100 lbs.)	Light or sedentary work.	2100	67								
	Moderate work	2500	67			20 to 30	3000 to 4000	1·0 to 2·0		50	
	Very hard work	3000	67								
	Pregnancy	2100	101		1·5						400 to 800
	Lactation	2700	112		2·0						
Children	under 1 year	100/Kg.	3·5/Kg.								400 to 800
	1 to 3 years	900	3·5/Kg.								
	3 to 5 ,,	1200	3·5/Kg.								
	5 to 7 ,,	1400	3·0/Kg.		1·0	10	3000	0·5		30	
	7 to 9 ,,	1700	2·5/Kg.		to	to	to	to		to	
	9 to 12 ,,	2000			1·5	30	4000	1·0		50 and over	
Adolescents	12 to 15 ,,	2400									
	15 to 21 ,,	2400	2·0/Kg.								

See note (4) following the table. — See footnote (10) following table. —

N.B.—The estimates of the protein requirements of children and adolescents are given in terms of grammes per kilogram because adequate data about average weight in the various age groups were not available to the Sub-Committee.

Notes on Table 1.

(1) The term 'net calories' means the energy available from the food actually assimilated.

(2) Additional calories for moderate and heavy work have been provided for, in accordance with the recommendations of the Technical Commission on Nutrition of the League of Nations Health Organization.

(3) Proteins of animal origin are generally superior in biological value to vegetable proteins. It is, therefore, desirable that some animal proteins should be included in the diet. Various estimates have been made of the desirable proportion of animal to vegetable proteins, e.g. 1:1, 0·5:1 or less. These are however not based on a fully satisfactory scientific foundation. Some animal protein should, however, be included in the diet. The diet given in Section III contains about 29 gms. of animal proteins equivalent to about 29 per cent of the total protein.

(4) Fats must be included in a balanced diet but there is no exact knowledge at present available of the quantity required ; hence no figures have been included in the table. Fats possess the advantage of yielding more than twice the energy obtained from carbohydrates or proteins. It is the general experience of nutrition workers that, even in a temperate climate, there is a tendency towards a higher consumption of fats in winter than in summer. A liberal consumption of fats can be advocated on the grounds that some of them act as vehicles for fat soluble vitamins and thus may provide these nutrients to the body in appreciable quantities.

(5) Figures for carbohydrate requirements are not given in the table. If the constituents listed in the table are obtained from a variety of natural foodstuffs adequate amounts of carbohydrate will be obtained.

(6) Equivalents of 1 milligramme of various vitamins in international units are shown below :

1·0	milligramme	β-carotene	=	1666 I.U. Vitamin A
1·0	,,	Vitamin A	=	3300 I.U.
1·0	,,	Thiamin hydrochloride	=	333 I.U. Vitamin B_1
1·0	,,	Ascorbic acid	=	20 I.U. Vitamin C
1·0	,,	Calciferol	=	40000 I.U. Vitamin D

(7) Vitamin A requirements can be met by preformed vitamin from animal foods, and by provitamin A (carotene) present in some foods of plant origin. When the latter form the bulk of the source of the vitamin, a higher level of intake is necessary than when preformed vitamin A is the source of supply. In Indian diets provitamin A is the main source of vitamin A activity. The figure given in the table is intended to cover vitamin A requirements in terms of Indian food habits.

(8) Vitamin D is undoubtedly necessary for older children although no definite figure can be given at present. Exposure to the ultraviolet component of sunlight leads to the formation of vitamin D in the skin and thus may supply a part of vitamin D requirement. No data are available about the contribution to vitamin D requirements from this source in tropical and subtropical countries.

(9) The information about the availability of iron from different foodstuffs is incomplete. Hence a figure for total iron intake higher than the usually accepted standard is included in the table.

(10) The human requirements of riboflavin, nicotinic acid and other members of vitamin B_2 complex have not yet been placed on a fully satisfactory basis and hence are not included in the table. These vitamins are, however, essential for human nutrition. A few quantitative estimates of requirements have been made, e.g. from 2·2 to 3·3. mgs. of riboflavin and 15 to 23 mgs. of nicotinic acid for adult men. Future research in India and elsewhere should be directed to placing this problem on a firm scientific basis.

(11) There are several other minerals which are essential in nutrition, e.g., iodine, magnesium, copper, manganese, etc. In general, if a diet is well-balanced and is adequate in respect of other better known essential nutrients it can be assumed that it will supply such minerals in adequate quantities.

(12) Allowance has been made for the unavailability of a certain proportion of most of the constituents in mixtures of foodstuffs, as also for the possibility of destruction through methods of preparation.

III. Requirements of foodstuffs.

The following table contains suggestions for the daily consumption of foodstuffs per adult man. The figures take into account the dietary habits of the people in different parts of India. Such a diet will provide in adequate measure the essential nutrients listed earlier.

Table II. Composition of a balanced diet.

(*Adequate for the maintenance of good health*)

	oz.
Cereals	14
Pulses	3
Green leafy vegetables	4
Root vegetables	3
Other vegetables	3
Fruits	3
Milk	10
Sugar and jaggery	2
Vegetable oil, ghee, etc.	2
Fish and meat	3
Eggs	1 egg.

1. *Cereals.* The type of the cereal forming the staple article of diet will vary according to locality. This variation will, however, cause little appreciable disturbance in the nutritive value of the diet, for the non-cereal portion of the diet as advocated provides most of the essential nutrients in requisite amounts.

2. *Pulses.* Pulses have a high protein content and are an important source of protein in Indian diets. They are particularly valuable in adding to the protein content of vegetarian diets. In addition they are a useful source of thiamin, and certain vitamins of the B_2 group. While pulses are at present included in all Indian diets, an increased intake is desirable from the nutritional standpoint.

3. *Vegetables.* Leafy vegetables are rich sources of vitamin C and provitamin A ; besides, they provide a certain amount of roughage. Certain leafy vegetables are particularly rich in calcium. Most vegetables are good sources of minerals.

4. *Fruits.* There are certain fruits, which are rich sources of provitamin A and vitamin C. Fruits also supply minerals. It is desirable that the quantity of fruits indicated in the table should be made up by such vitamin-rich varieties as can be found in any particular season. Fruits of high vitamin content include amla, citrus fruits, mango, papaya, guava, etc.

5. *Fats and oils.* The quantity of total fat in a diet made up according to the table will be about 90 gms. Under the heading *fats* in the table is included the fat or oil used for cooking and flavouring the food. As much of this as possible should be butter or ghee.

6. *Fish, meat and eggs.* These foodstuffs are excellent sources of proteins of high biological value and good sources of vitamins of the B_2 group. Egg is rich in vitamin A and is the only natural foodstuff, besides milk fat, supplying appreciable amounts of vitamin D.

7. *Sugar and jaggery.* Sugar and related products are used mainly as sweetening agents. They thus increase the palatability of foods and also contribute to the energy value of the diet. Jaggery also adds to the mineral constituents of the diet.

8. *Condiments and spices.* These accessory foodstuffs are not included in the diet table. Most of them are used for flavouring foods. Some of them contribute in appreciable amounts essential nutrients even in the small quantities in which they are used. Their value in improving the palatability of the diet is to be particularly stressed, and as such their use in moderate quantities is desirable.

9. *Milk and milk-products.* In table II the requirement of an adult has been placed at 10 ounces per day. We are not satisfied with this low figure ; it may, however, be taken as a practicable objective to be reached within a short period. When conditions improve the figure for milk requirement will have to be increased, and brought in line with the commonly accepted standard of 20 ozs. per adult per day. It appears that in certain parts of the country such a figure has already been reached. The Committee feels that in future care should be taken to see that the level of intake in such areas is not lowered. During infancy and childhood the requirements of protective foodstuffs, particularly

milk, are greater than those advocated for adults (Table II), e.g., nutrition workers recommend a daily allowance of about 40 ounces per child of 1 to 6 years. It is necessary to stress therefore that *in considering the distribution of the available milk supply the needs of infants, growing children and pregnant and nursing women should receive a high priority.*

10. *Requirements of pregnant and nursing women.* During pregnancy and lactation a woman needs more protein and minerals. The extra protein can be obtained by substitution of the cereal portion of the diet by more milk, fish, meat and eggs, particularly milk, and in case of vegetarians by a further additional provision of milk. This would also ensure the necessary additional supply of minerals.

IV. Food production.

The diet given in table II indicates the requirements of an adult man in terms of foodstuffs. The requirements of women and children of various ages can be deduced from those of an adult man by applying a suitable scale of family consumption co-efficients in which the needs of the various age groups are expressed as fractions of those of an adult man. For practical purposes the number of adult consumption units per 100 of the population can be taken as 80, which is in conformity with the present method of calculation adopted by food departments. The present population in India is approximately 400 millions, and on this basis some 320 million consumption units have to be provided for. In long-term planning, however, account must be taken of the growth of the population. The long-term production targets given in table III represent the requirements of 400 million adult males or consumption units instead of 320 million which is approximately the present figure. These estimates are for foodstuffs meant for human consumption only exclusive of the requirements of livestock and seed and of wastage.

Table III. Estimated annual requirements of foodstuffs for human consumption.

	Million tons	
1. Cereals	57	
2. Pulses	12	
3. Vegetables		
(1) Green leafy	16	
(2) Non-leafy including tuberous vegetables	24	
4. Fruits	12	
5. Sugar and jaggery	8	
6. Fats (oils and ghee)	8	
7. Milk	46	
8. Fish and meat	9	Assuming that 30% of the population is vegetarian and would not partake of these foods.
9. Eggs	102000 million eggs	

In the case of certain protective foods, it is incorrect to reckon the needs of children as lesser fractions of those of adults. Thus children in general need *more* milk than adults and hence the figure for milk requirements is an underestimate. Since, however, the estimate is, far in excess of the existing productive capacity of the country, it is scarcely necessary, at this stage, to raise it further by allowing for the higher requirements of children.

Any estimates of the increase required in the production of the various foodstuffs must be based on accurate knowledge of present food production. It is now generally recognised that agricultural statistics in India are inaccurate and that their improvement is essential for satisfactory food planning. Steps are already being taken to remedy the position.

The total quantity of food grains (rice, wheat, barley, maize, jowar, bajra, other millets and gram) available annually for consumption in India has recently been reckoned at 60 million tons.* From this figure should be deducted 3·5 million tons of gram which should, strictly speaking, be included in the category of pulses. From the remainder a deduction of 12½ per cent will have to be made to cover seed requirements and wastage in storage. Thus the net quantity of cereals annually available for human consumption would be about 49 million tons, a figure which is below the estimated requirements. The deficit is not as large as it would have been had the calculations been made, as they have been made by others, on the basis of 16 oz. of cereals per adult per day. The fact that cereal requirements, as part of a programme which calls for the production of other non-cereal foods far in excess of the present level, are evaluated in this Report at 14 oz., makes it essential to emphasise strongly that 14 oz. of cereals per day are adequate only if the diet contains the other foods listed in Table II in sufficient quantity. At present production of the latter is highly insufficient and enormous effort will be needed over a period of years to bring it to position approaching adequacy. Since the Committee are informed that it is more immediately feasible to increase the production of cereals than that of other foods up to the required standard emphasis must be laid on more cereals as part of short term production policy. Further there is a serious shortage of rice in the country if requirements are assessed in terms of the demand of those who prefer rice to other cereals.

Of the various cereals, rice and wheat are of course the most important, although barley, jowar and bajra are consumed as staples in some parts of India. Ragi (nachni, morua, *Eleusine coracana*) is a cereal which forms a staple article of diet of millions of poor people in Mysore, Madras and Konkan. This cereal is rich in calcium and its importance and value as a foodstuff should be more generally realised.

Apart from production statistics, valuable information about the diets consumed by different population groups can be obtained by means of diet surveys. A considerable number of diet surveys have already been carried out in India, but there are numerous areas in which such inquiries have not been made. Most of the surveys were carried but before the present difficult food situation arose and knowledge of the effect of the food situation on dietary habits is in general lacking. It is essential, in connection with food policy, to have accurate information about the kind and quantities of various foods which are actually being consumed. Arrangements should be made for surveys to be carried out by Food and Health Departments with the collaboration and assistance of nutrition workers.

* Memorandum on Agricological Development in India prepared for the Advisory Board of the I.C.A.R., 1944.

V. Practical considerations.

We are not at present in a position to give provincial production targets. Since India should be regarded as a single unit in the matter of food supplies, all-India and regional food policies must be closely coordinated. Certain areas are surplus in certain foods, others deficit. Some kinds of food can be produced more easily in certain parts of the country than in others. The satisfactory transport and distribution of food are as important as its production. Policy with regard to imports and exports must be given due consideration. Cash crops have to be balanced against food crops. The price of different foodstuffs is of primary importance, and farm machinery, man power, food preservation, processing and storage are among the questions intimately connected with food policy. The problem is far from the simple one of calculating the quantity of food required on an all-India or provincial basis, and adjusting agricultural production to meet these requirements.

Production targets based on satisfactory estimates of existing and potential production and the nutritional requirements of the population should be set up by provinces and states in close collaboration with the government at the centre. In carrying out this task due allowance must be made for all the factors in the situation including those referred to above. Food and agricultural authorities should in all their activities strive towards one goal—the provision of a satisfactory diet for the entire population. As regards immediate policy emphasis may be laid on an increase in the production of fish and vegetables, the education of the public to popularise alternative cereals to rice, to schemes for covering the special needs of "vulnerable groups", e.g. school-feeding, the provision of milk to expectant and nursing mothers and young children, etc. Other immediate measures are considered in the Nutrition Advisory Committee's report of March 1944. As regards long-term policy, the objection may be made that the production targets given in this report are outside the range of practical possibilities. We may agree that they can be attained only by untiring effort over a considerable period of years. Apart from this, they indicate the *direction* in which advance should be made. A beginning should be made immediately to push production in this direction.

It is the duty of the nutrition worker to insist that one of the principal objectives of economic and agricultural policies is the provision of an adequate and well-balanced diet and to make it abundantly clear what is meant by the latter. How and where the necessary foods are to be produced are not his direct concern.

NUTRITION ADVISORY COMMITTEE, INDIAN RESEARCH FUND ASSOCIATION.

REPORT OF THE SOYA-BEAN SUB-COMMITTEE NOVEMBER 23rd, 1944.

(1) There has been considerable controversy in India for a number of years about the question of encouraging the production and use of soya-bean as an important article of diet. On the one hand, enthusiastic advocates of soya-bean have insisted that in this pulse lies the solution of the problem of nutrition in India. On the other hand, doubts were expressed whether the nutritive value of soya-bean, compared with that of common Indian pulses, is such as to make the effort of extending its cultivation and consumption worth while.

The Soya-bean Sub-Committee was constituted in 1941 to provide an answer to the question at issue, based on scientific data. At its first meeting it drew up a scheme of conjoint and coordinated research which has been carried out in the following laboratories :—

The Biochemical Laboratory, University of Dacca, under Dr. K. P. Basu.

The Seth G. S. Medical College, Bombay, under Dr. V. N. Patwardhan.

The University Chemical Laboratories, Lahore, under Dr. Bashir Ahmad.

The Nutrition Research Laboratories, Coonoor, under Dr. W. R. Aykroyd.

The lines of investigation proposed by the Sub-Committee were followed on uniform lines in the participating laboratories. The results were scrutinized by the Sub-Committee in 1942 and 1943, and a further programme of research for the following year elaborated. A very considerable bulk of data has been obtained, and in general the results obtained by the different workers are in good agreement. The most important conclusions may be briefly summarised as follows :—

(1) The biological value of the proteins of cooked soya-bean is approximately the same as that of certain common Indian pulses, also cooked. The pulses investigated were Bengal gram (*Cicer arietinum*), green gram (*Phaseolus radiatus*) and red gram (*Cajanus indicus*).

(2) Since soya-bean has a higher protein content than other pulses, the *total* quantity of protein assimilated from a given amount of soya-bean will be greater than that assimilated from an equivalent quantity of other pulses.

(3) The supplementary effect of soya-bean on the growth of rats on a poor rice diet is of the same order as that of the other pulses.

(4) When the poor rice diet is supplemented by calcium and B vitamins the growth of rats on the diet is enhanced. The effect of soya-bean given as an addition to the rice diet supplemented in this manner is approximately the same as that of the other pulses.

Other experiments have included an investigation of the growth response of school-children to supplements of soya-bean and red gram, and the effect of parching and autoclaving on the biological value of soya-bean and other pulse proteins. In the school-feeding experiment, no statistically significant

difference was observed in the weight increments of the groups fed on the two kinds of pulses during the experimental period. Parching and autoclaving enhanced the biological value of soya-bean proteins; but the difference in value between soya-bean and other pulses treated in this manner was not of an important order.

The animal experiments and the single experiment with human beings thus indicate that soya-bean as a protein supplement is not superior to some pulses in common use. The experiments on rats with the poor rice diet suggest that a diet based largely on rice is not markedly deficient in good protein. It appears, therefore, that the higher total protein content of soya-bean does not give it any special advantage over the other pulses as a supplement to a rice diet. In the case of poor rice diets, deficiency of calcium and of the B group of vitamins is more important than deficiency of protein.

(2) In view of these facts and conclusions, the Sub-Committee is of the opinion that the nutritive value of soya-bean, in comparison with that of other common Indian pulses, is *not* such as to justify, from the standpoint of human nutrition, the immediate encouragement of the production and consumption of soya-bean on a wide scale in India.

The Sub-Committee realises that there may be varieties of soya-bean which have a nutritive value higher than that of those studied in the present investigation. Further study of different varieties can with advantage be carried out. Some evidence has been obtained that Bengal gram is slightly superior in nutritive value to other Indian pulses and the Sub-Committee considers it worth while to draw the attention of agricultural authorities to this pulse as a useful article of diet whose production could with advantage be extended.

(3) *Publication of results.* It is recommended that a conjoint report should be published by the Sub-Committee. This will describe in detail the work carried out under its auspices.

(4) *Future work.* It is not proposed to continue work in Coonoor and Dacca during 1945-46. In Bombay the amino-acid make-up of soya-bean will be studied, as part of an investigation of amino-acids in a series of proteins. In Dacca further work will be carried out on the nutritive value of different varieties of soya-bean proteins and on the amino-acid composition of this pulse. It is also proposed to study the value of soya-bean milk supplemented by calcium and vitamins, as a substitute for animal milk. These investigations will be part of the work of the nutrition research units in Bombay and Dacca.

The Sub-Committee feels that, except for the publication of a full technical report, its task is for the time being completed.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on the 28th November, 1944, the following members being present :

Dr. K. V. Venkatraman (*Chairman*).
Lieut-Colonel L. A. P. Anderson.
Dr. A. N. Goyle.
Dr. S. R. Pandit.
Dr. R. M. Mathew.
Lieut.-Colonel S. S. Sokhey, (*Secretary*).

The following were also present :

Lieut.-Colonel C. L. Pasricha.
Major B. N. Khan.
Dr. P. V. George.

The reports of the enquiries in progress during the year were considered.

(i) Plague enquiry under the Director, Haffkine Institute, Bombay.

(a) Field trials of sulfathiazole and sulfadiazine.

The trial was carried out at the Infectious Diseases Hospital at Poona and lasted from middle of November, 1943 to the middle of February 1944, to find the comparative therapeutic value of sulfathiazole and sulfadiazine. Admissions were treated in strict rotation with the two drugs and no selection was made. Both the drugs were used in a dosage of 10 gms. the first day and 6 gms. a day on subsequent days of treatment. In the case of sulfathiazole a blood concentration of 5–10 mg. and in the case of sulfadiazine of 10–15 mg. per 100 ml. was attained. 182 cases were treated in all, out of which 26 who died within 24 hours of admission were omitted. Taking only the cases that had plague septicaemia as determined by blood culture at the commencement of treatment, the following results were obtained :

Drug.	Number of cases.	Number of deaths.	Case mortality per cent.
Sulfathiazole ..	40	15	37.5
Sulfadiazine ..	43	9	20.9

Though the difference in the results of the treatments is not statistically significant, clinically the cases treated with sulfadiazine seemed to do better. It is noted that no serious toxic symptoms were observed in the cases treated with either of the two drugs ; sulfadiazine however produced fewer toxic reactions.

(b) Plague vaccine : Avirulent live vaccine-Experimental evidence was produced to show that for such a vaccine to have any protective power at all it must be prepared from strains which have a residual virulence and are capable of multiplying in the body. These observations indicated that for such vaccine to be used they will have to be made fresh and would suffer from the possible risk of enhancement of virulence.

(ii) A new plague vaccine was prepared in modified acid hydrolysate of casein (Muller and Johnson). The medium gives a negative bivret reaction and negative intradermal skin test to protein. After two weeks incubation at 28°C in this medium, the growth was killed with 0.1% formalin and phenyl mercuric

nitrate 1 mg. per 100 ml. added as preservative. The protective power of the new vaccine was twice as great as the original Haffkine vaccine and slightly more than agar vaccine (grown at 37°C.). Its toxicity was low and keeping properties better than the phenol preserved agar vaccine.

The Committee note that the results of field trials with the sulfonamides are encouraging and consider that they should be extended using further compounds like methyl salt of sulfadiazine. The work on vaccine has thrown valuable light on the relationship of the virulence of the strain used in vaccine production to the protective value of the vaccine. It has also yielded a new vaccine which appears to be an improvement on the previous vaccines in protective power, lowered toxicity and better keeping properties.

The Committee is of the opinion that this work should be continued during 1945-46 and recommend that a grant of Rs. 11,754 asked for may be allotted.

2. Plague enquiry under the Director of Public Health, Madras.

The Committee note with interest the work done on plague control in certain villages of the Hosur Taluk. The material obtained during this work has been availed of for the purposes of investigation of the distribution of the several species of fleas and their relation to the transmission of plague. It is noted that braziliensis predominated on domestic rats and astia on field rats and the peak of human plague followed the braziliensis. As regards Sylvatic Plague it is noted that the Plague infected gerbilles were obtained from the vicinity of a plague infected village and afford no evidence of the existence of Sylvatic Plague.

The Committee recommend the continuance of the contribution of Rs. 3,000/- asked for on condition that the Madras Government continue their contribution.

3. The Committee considered a letter dated November 1, 1944, from the Chairman, Indian Pharmacopoeial List Committee, regarding the advisability of including plague vaccine in the official supplement to the B. P. and decided that for the present this product should not be included.

Report of the Committee on Anti-Smallpox Vaccination in British India held in the Committee Room A, Imperial Secretariat (North Block), New Delhi at 10 a.m. on the 22nd November, 1944.

MEMBERS PRESENT :

1. Dr. A. C. Banerjea, C.I.E., Director of Public Health, United Provinces, Lucknow. (*Chairman*).
2. Lieut.-Col. H. W. Mulligan, I.M.S., Director, Central Research Institute, Kasauli.
3. Major C. L. Pasricha, I.M.S., Professor of Pathology and Bacteriology, School of Tropical Medicine, Calcutta.
4. Khan Bahadur Dr. A. H. Butt, Director of Public Health, Punjab, Lahore.
5. Major M. Jafar, I.M.S., Director of Public Health, Bengal, Calcutta.
6. Major A. N. Duggal, I.M.S., Additional Director of Public Health, Bihar, Patna.
7. Major T. D. Ahmad, Director of Public Health, Assam, Shillong.
8. Dr. S. R. Pandit, M.B.B.S., B.Sc., D.B., Director, Pasteur Institute, Shillong.
9. Dr. R. K. Goyal, M.B., D.Sc., Ph.D., Central Research Institute, Kasauli.
10. Rai Sahib Dr. B. S. Yajnik, M.B.B.S., D.P.H., Officer on Special Duty (Public Health), Office of the Public Health Commissioner with the Government of India (*Secretary*).

Lt.-Col. L. A. P. Anderson, I.M.S. (Retd.), Officer on Special Duty (Blood Transfusion) and Khan Sahib Dr. K. S. Shah, Superintendent, Punjab Vaccine Institute, Lahore, also attended the meeting.

The Committee discussed vaccination against smallpox in all its aspects on the basis of a note prepared by Rai Sahib Dr. B. S. Yajnik.

It was not possible in the time available to do more than formulate recommendations in general terms. These are given below :—

1. Primary vaccination in infancy should be compulsory. Revaccination should be compulsory at the age of 5 years as a routine and for all age groups in the presence or under the threat of epidemic smallpox.

For both primary and revaccination the committee recommends vaccination by three insertions equivalent in all to a linear insertion of one inch. The Committee considers that the rotary lancet in almost universal use throughout India is a convenient, popular and satisfactory instrument in the hands of Public Vaccinators and that, when this instrument is used, three circular insertions should be made. In making this recommendation the Committee have taken into consideration the extreme rarity with which postvaccinal encephalitis occurs in India and the desirability of imparting optimum immunity against smallpox with the least possible trauma and inconvenience to vaccinated persons.

2. Existing legislation is in some respects out of date and should be amended to make provision for the recommendations made in para 1 above.

3. The state of immunity to smallpox of the general population of India brought by vaccination, as at present practised, has not been accurately determined. A statistical investigation in selected areas of different provinces is required. Such investigations should be carried out in accordance with a prearranged plan so that the data made available in this way would be capable of

accurate statistical analyses. The preparation of a suitable plan for this purpose should be entrusted to a special committee, suitable members of which would be Dr. A. C. Banerjea, Major Pasricha, Dr. B. S. Yajnik and a statistical expert such as Mr. S. Swarup of the Punjab or Dr. P. Chaudhury of Bengal Public Health Department. Directors of Public Health of the provinces should be invited to undertake investigations in selected areas in accordance with the plan formulated in this way.

4. The Committee recognises that compulsory vaccination as recommended in para 1 above and the collection of accurate data on this subject demands *inter alia* more accurate registration of births and deaths throughout India. For this, and other obvious reasons the Committee considers that the whole system of registering vital statistics in India requires to be overhauled.

5. The Committee recognises the necessity for obtaining the highest possible degree of immunity against smallpox and considers that with this end in view every effort should be made to ensure the use of vaccine lymph of maximum potency manufactured under ideal conditions and stored and distributed in a manner suitable to the preservation of these qualities. The Committee is not satisfied that the vaccine lymph at present available for general use in India is the best that can be evolved and they recommend that the whole subject be reinvestigated. In the Committee's opinion the investigation of the possibility of producing a more satisfactory immunising agent against smallpox is one which might suitably receive attention of the Indian Research Fund Association.

The Committee recommends that a specific standard for the most suitable available immunising agent against smallpox should be laid down under the Drugs Act (1940) and the Rules under this Act.

6. Pending the conduct of an extensive investigation such as that visualised in para 5 above, the Committee recommends that strict attention should be paid to the distribution of vaccine lymph in such a manner as to maintain the potency at the highest possible level during transit. In our present state of knowledge this means in effect that the lymph should be kept as cool as possible and to this end special methods of packing and distribution should be arranged, specially in regard to the supply of lymph to remote and isolated areas. Periodical checks should be made of the potency of lymph in actual use in field.

7. The Committee reviewed the question of recruitment, training, emoluments and other service conditions of vaccinators employed in various provinces. The Committee considers that the vaccinators at present employed have insufficient basic qualifications and training and that in order to achieve a higher standard of vaccination it will be necessary to raise the status of Vaccinators to that of the Sanitary Inspectors. Part-time or licensed Vaccinators should not be employed. In future, vaccination should be entrusted only to the qualified Sanitary Inspectors specially trained in vaccination technique. The work of vaccinators should be subjected to periodical check by officers of public health departments and particularly by specially appointed Chief Sanitary Inspectors. In the Committee's opinion this service of Sanitary Inspectors and Chief Sanitary Inspectors should be provincialised. Suitable rate of pay for Chief Sanitary Inspectors would be 100–5–150 and for Sanitary Inspectors 60–2–80–4–100. The Committee considers that one qualified Sanitary Inspector should be provided for every 25,000 population and one Chief Sanitary Inspector to every 10 Sanitary Inspectors. The Committee recognises the great difficulty in the way of appointing female vaccinators except in urban areas and recommends

that in order to make provision for the vaccination of females in the rural areas women doctors, midwives, female health visitors, school teachers and welfare workers should be specially trained in the technique of vaccination.

8. The Committee desires that the above recommendations should be brought to the notice of the Health Survey and Development Committee who, it is understood, are likely to consider the whole subject of vaccination against smallpox in the course of their deliberations.

Agenda for the discussion on Anti-Smallpox Vaccination in British India held in the Committee Room A, Imperial Secretariat, North Block, New Delhi, at 10 a.m. on the 22nd November, 1944.

1. The consideration of existing legislation.
2. Collection of statistical data with regard to the immunity conferred by vaccination.
3. The preparation of vaccine lymph—e.g. strains used, potency, methods of manufacture, methods of storage, freedom from toxicity, contamination, etc.
4. Distribution of vaccine lymph from depots—e.g. conditions of transit, time out of cold storage before use, methods of transportation used by vaccinators, etc.
5. The employment of suitable and reliable vaccinators—e.g. part-time employees, miserable emoluments etc.
6. Technique of vaccination—e.g. how carried out, number of insertions, etc.
7. Supervision of vaccination—e.g. checking system on the activities of the vaccinators employed, etc.

B.

POINTS SUGGESTED FOR DISCUSSION UNDER THE ABOVE AGENDA :—

Item No. 1. Existing legislation :—

(a) To consider the desirability of amending the Vaccination Act (No. XIII of 1880) and the rules made thereunder with a view to provide for more efficient legal support to vaccination all over British India and also to bring it up to date in conformity with the present scientific procedures.

(b) To consider suggestions, if any, in the draft provisions applicable to the production of vaccine lymph (vaccinia) made under the Drugs Act, 1940, circulated for objections with Notification No. 54-3/40-H, dated the 14th April, 1944.

Item No. 2. Statistical data :—

(a) To consider the desirability of collecting necessary statistical data regarding the present vaccinal immunity status of the general population in certain selected areas.

(b) To consider suggestions, if any, regarding collection, recording and compilation of suitable statistical data with a view to determine the quantity and quality of work done by the vaccination staff

and the effect of vaccination on the control of smallpox epidemics in India.

Item No. 3. Preparation of vaccine lymph :—

To consider the question of improving antigenic power, purity and potency of vaccine lymph manufactured in India with special reference to :—

(i) Use of proper strain of vaccinia virus,
(ii) Rejuvenation of seed virus,
(iii) Cold storage,
(iv) Purification processes,
(v) Purity tests (freedom from extraneous and harmful germs).
(vi) Toxicity or pathogenicity tests,
(vii) Potency tests, and
(viii) Field tests.

Item No. 4. Distribution of vaccine lymph :—

To consider improvements in the existing arrangements for the distribution of vaccine lymph to vaccinators as regards :—

(i) Mode of packing,
(ii) Cooling arrangements during transit,
(iii) Postal facilities,
(iv) Prompt use by vaccinators.

Item No. 6. Technique of vaccination :—

To consider suggestions, if any, in the present day vaccination technique regarding :—

(i) Handling of vaccine lymph by vaccinators,
(ii) Aseptic precautions,
(iii) Number, size and depth of insertions,
(iv) Type of instruments used,
(v) Care of developing pustule,
(vi) Inspection and recording of results.

Items No. 5 & 7. Vaccination staff :—

To consider improvements in the organisation of vaccination staff regarding :—

(i) Training,
(ii) Recruitment,
(iii) Grades and promotions,
(iv) Supervision of their work,
(v) Duties during vaccination season and off season,
(vi) Appointments of female vaccinators.

ANTI-SMALLPOX VACCINATION IN INDIA.

(A NOTE ON THE SUGGESTED POINTS FOR DISCUSSION)

1 (a). There are several vaccination Acts such as the Bengal Vaccination Act, 1880, Bombay Vaccination Act, 1877, Karachi Vaccination Act, 1879, Vaccination Act, 1880, etc. which are applicable to the different areas. Vaccination Act No. XIII of 1880 is applicable to almost all the provinces excepting the three Presidencies of Madras, Bombay and Bengal, where similar Acts were

promulgated. These Acts were amended by local governments at different times and the main provisions of all these Acts are as follows :—

(1) That all children between the ages of six months and (if male) 14 years, (if female 8 years) residing in an area, where the Act applies, for more than 1 month must be vaccinated, (Sec. 9).

(2) That the parent or guardian shall be responsible for having this done, (Sec. 9).

(3) That, if in ill health, vaccination may be postponed, (Sec. 9).

(4) That a certificate of successful vaccination should be given (Sec. 11).

The Acts are applicable mostly to urban areas and require extension to rural areas where vaccination is still performed by persuasive means. They should be extended throughout British India. Besides, no specific provision has been made for compulsory revaccination, a procedure which is very essential to cope with the prevailing menace of smallpox in India.

Compulsory revaccination is in force only in the province of Madras where the Madras government has taken steps to promulgate it under the Local-Self Government Act, as no provision exists to enforce compulsory vaccination under the Vaccination Act. Other provinces cannot arrange for compulsory revaccination without an amendment of the Vaccination Act. Temporary regulations can, however, be promulgated under the Epidemic Diseases Act, but that will not do, as it will not be a permanent measure.

An interval between revaccination under different conditions is to be prescribed. Madras practice is to have it after 7 years in the city of Madras and after ten years in other areas. In the British army this interval is now 5 years in the case of forces remaining in the United Kingdom and 3 years in the case of forces over-seas. The recommendation of the Ministry of Health suggests revaccination every two years, if vaccination is performed with three insertions, and every year if only single insertion vaccination is done. The period of 7 years, although not ideal in all cases, seems practicable, and if, prescribed will raise the herd immunity to a high level. According to Army Regulations in India a sufficient protection means successful vaccination within the last two years.

Rules made under the Act should lay special stress on the preparation of a register for unprotected children (children available for vaccination), recording of results, date of inspection, recording of revaccination results. No vaccination operation is really unsuccessful, provided a good potent lymph is used. These points are discussed under item 6 of the agenda and the existing rules may require modification as a result of the discussion.

Certain provisions in the Vaccination Act like maintenance of private licensed vaccinators (Sec. 8) and use of human lymph for vaccination (Sec. 15) may now be removed. Inoculation (variolation) is no longer practised. So section 6 prohibiting this practice is actually not required, but reports are often received about inoculation practised in the Nepal territory. So this Section can help us to prohibit the entry of inoculated persons provided the Act is extended to the whole of British India. Certain malpractices cannot be prevented under this Act. Recently a case has been brought to our notice under which a faqir following the vaccination technique applied human or cow's milk as a ' sure ' protection against smallpox. This type of crude non-specific protein therapy as a prophylactic against smallpox or other malpractices require consideration.

1 (b). Provisions applicable to the production of vaccine lymph (vaccinia) are described on page 84 of the Notification No. F. 54-3/40-H, dated the 14th April, 1944. These rules are primarily based on the Therapeutic Substances Regulations (May 31, 1927) of England.

Recently some amendments have been made by the Ministry of Health in the Therapeutic Substances Regulations of England and the standard for purity and potency of vaccine lymph has considerably been modified. The important modifications made therein relate to the following :—

(1) Total number of living bacteria or other extraneous organisms can be tolerated now upto 20,000 per c.c. instead of 5,000 per c.c.

(2) Tests for the presence of B. anthracis and B. coli are now to be undertaken.

(3) The minimum amount of vaccine lymph subjected to various tests has now been prescribed.

(4) Tests are to be carried on to indicate the absence of Cl. tetani only instead of all types of gas-producing anaerobes.

(5) Tests are to be carried on to indicate absence of only beta-heamolytic streptococci instead of all the types of streptococci.

(6) In the potency test the use of guinea-pig is no longer allowed and a rabbit is the only animal suggested for the purpose. The standard of potency remains unchanged.

It may be desirable to take advantage of these amendments and modify the draft rules under the Indian Drugs Act now under consideration of the government.

Changing the standard for colony count from 5,000 to 20,000 per c.c. will be very helpful and may allow considerable reduction in the storage period and thus ensure better potency. The standard of one in thousand dilution for potency test on rabbits seems to be rather low for India and we should try to have a higher standard of, say, one in two thousand or one in four thousand dilution because in tropical countries deterioration of vaccine lymph during transit is rather rapid. Best indication is, however, given by a field test on children. It should be prescribed under the rules because it indicates the quality of vaccine lymph under actual field conditions. A statisfactory report indicating 95—100% case success or over 90% insertion success rate after trial on at least 50 children (primary cases) at five or more vaccination stations in different parts of a province may be prescribed for the purpose. Such field tests should invariably be performed by different vaccinators four weeks before the actual time of despatch.

No toxicity or pathogenicity tests are prescribed but such tests on guinea-pigs are undertaken in certain provinces. They may also be prescribed because such tests offer the greatest possible safeguard against any pathogenic organism in vaccine lypmh undetected by other tests. Such tests will introduce a sense of security and ensure the use of 'safe' lymph.

2. (a). No reliable investigation has been made in India regarding degree of protection afforded by vaccination, duration of immunity, immunity status of general population (herd immunity). Work has been done by individual workers on limited scale in restricted areas. It is desirable to collect, record, compile and evaluate necessary statistical data concerning protection afforded

by vaccination. Data regarding revaccination results and vaccinal status of smallpox cases should be collected and the effect of vaccination on the incidence and mortality due to smallpox determined.

Outbreaks of smallpox depend upon the relative increase in the number of susceptible persons in a locality. Unprotected children and people who had lost their protection due to lack of revaccination are responsible for the periodical outbreaks of smallpox. An idea of the number of susceptible persons can be had if necessary statistical data outlined above are collected.

2 (b). The quantity and quality of the work done by the vaccination staff can be determined if insertion success rate is also recorded along with the case success rate. Insertion success rate indicates the quality of work done by vaccinators and the case success rate shows the quantity. Proper preparation of the register of unprotected children is very necessary to find out whether all the children available for vaccination have really been vaccinated or not. Besides, proper recording of revaccination results as 'immediate' 'accelerated' or 'typical primary' should be done by vaccinators. This will mean frequent inspection of the vaccinated children by the vaccinators. Vaccinal history of smallpox cases should be investigated in as many cases as possible. Severity of the disease, age and sex of the patient, period elapsing since the last successful vaccination or revaccination, presence or absence of scars and other relevant particulars must be collected to find out the quality of work done in particular areas during the last few years. Improvement in registration of vital statistics is of the utmost importance in the vaccination campaign.

3 (i). It is very common to blame the quality of vaccine lymph for all the deficiencies. It is rather difficult for the people to appreciate that no immunizing procedure, however perfect the agent and the technique employed, would guarantee 100 per cent immunising protection. The quality of vaccine lymph manufactured in India has been questioned, but the evidence is not entirely conclusive. A detailed investigation is desirable on the various aspects of the manufacture of vaccine lymph. Deficiencies in vaccination in India have been reported and noticed by many workers, but how far the quality of vaccine lymph supplied by different institutes, is responsible for it, is not known. It may be that defective or wrong technique of vaccination may be responsible for this. Some workers have put forward evidence in favour of inferiority of certain vaccine samples obtained from Indian institutes as compared with the imported strains. Imported samples of vaccine lymph have shown better "takes" than the locally manufactured samples. Some have raised the question of the difference in strains and have suggested that the strains used in India may be particularly ineffective in producing immunity. This type of work done in India requires to be undertaken on thorough lines under proper control, because experience of other workers has shown that parallel vaccination experiments on a large number of persons have given almost identical results on the local or imported samples. Serological tests have shown that the strain used in India is antigenically not different from that produced from variola. Cross immunity tests and neutralization tests have also not shown any appreciable difference between imported and local samples of vaccine lymph.

Most surprising thing is that some workers have found Indian samples of vaccine lymph to be inferior to imported ones, while others have found that Indian samples are much superior to British samples. Cases have been reported where Indian samples have produced successful results in persons who showed poor revaccination results with British vaccine lymph. Really speaking the

whole subject requires thorough investigation as the varying results reported by different persons may very likely be due to difference in potency of samples at the time of actual use rather than to any basic antigenic variation or difference in strain. Now, as the quality of vaccine lymph has been questioned it must be thoroughly investigated.

3 (ii). The method of rejuvenation of seed virus in India varies in different provinces. Modified Nijland's (Java) method is used in most of the provinces, but some prefer Forester—Java method. The main thing is to use a rabbit in the series to produce rejuvenation. In some provinces detailed experiments were undertaken to find out the best method for this purpose. It has been found that introduction of a guinea-pig in the series lowers the quality. Series of animal passages used in various provinces are as follows :—

SEED VIRUS CYCLE.

U.P.	Buffalo calf—rabbit—bull calf—buffalo calf.
Madras.	Cow calf—rabbit—buffalo calf—buffalo calf—cow calf.
C.P.	Rabbit or a child is used in the series.
Bengal.	Cow calf—rabbit—buffalo calf—boffalo calf—cow calf.
Punjab.	Buffalo calf—rabbit—cow calf—buffalo calf.
Bihar.	Children, rabbits, buffalo calves and cow calves.
Bombay.	Rabbit.
Assam.	?
Burma.	Cow calf—rabbit—cow calf—buffalo calf—rabbit—cow calf.

3 (iii) Vaccine lymph requires to be stored at a very low temperature. In England it is stored at 10°C. Storage temperatures in various provinces are not known, but it is believed that it is not above 0°C. It is said that vaccine lymph, when stored at a temperature below 0°C, maintains its potency for long periods. When stored at temperatures from 0° to 5°C the potency may be expected to retain for three months, but when stored at temperatures from 5° to 10°C, the potency is retained for four weeks only. When stored above 10°C, the potency cannot be assured beyond seven days. It may be noted that frigidaire temperature is not suitable for storing vaccine lymph. The temperature in the freezing (ice) compartment of a frigidaire is also not low enough to allow storage for a few weeks.

3 (iv). The chief purification process adopted in various vaccine institutes consists of simple 'glycerination' (slow maturation). Pathogenic organisms get eliminated after a varying interval of time. Chloroforming of vaccine lymph is popular in certain institutes and the use of oil of cloves is also made at some places. The purity standard prescribed under the Therapeutic Substances Act is said to be followed rigidly by all the provinces. The period of storage necessary to effect the desired purification will be reduced considerably if the standard of 5,000 organisms per c.c. is changed to 20,000 per c.c.

3 (v). Tests for the absence of beta-haemolytic streptococci, Cl. tetani, B. anthracis and B. coli will have to be done if the rules under the Drugs Act are revised. At present tests are carried on for the absence of streptococci and gas-producing anaerobes. An official technique for these tests has been prescribed in British Pharmacopoeia, 1932. It is not known whether all the institutes

follow the same procedure. It seems that different techniques are adopted in various provinces.

3 (vi). Toxicity of pathogenicity test is not officially prescribed but this test ensures the harmless nature of vaccine lymph. It should therefore be prescribed and the details of the technique indicated in the British Pharmacopoeia may be followed.

3 (vii) According to the standard laid down under the Therapeutic Substances Act the vaccine lymph sample should produce characteristic vaccinia lesions on the prepared skin of a rabbit (guinea-pig is no longer allowed) in a dilution of 1 in 1,000. In some institutes rabbit test is not performed at all but reliance is placed on Cunningham's or King's test on calves or only on the field tests on a few children. The tests performed in various institutes are given below :—

Burma.	The test is performed on rabbits and the production of 3-4 vesicles per sq. c.m. with 1–1,000 dilution of lymph is the criterion for passing lymph.
U. P.	The test is performed on white rabbits and the formation of discrete eruptions with 1–4,000 dilution or more, and confluent with 1–1,000 dilution is the criterion for passing lymph.
Bombay.	The test is performed on rabbits and a semiconfluent 'take' is the criterion for passing lymph.
Madras.	The test is performed on calves. The number of vesicles divided by the length of the insertions must not be less than two per inch with 1–1,000 dilution.
Bihar.	Do. Do. Do.
Assam.	Do. but with only 1–1,000 dilution.
Bengal.	Do.
Punjab.	Animal test is not done.
C.P. & Berar	Do.

It will be seen that the technique of the potency test is not uniform all over India.

3 (viii). Field tests are usually done in all the provinces mostly by the public vaccinators. In most of the places the test is performed on three to five children, but in U. P. the test is performed on about 50 children in six to eight different places in the province. The standard for these tests varies. In most of the provinces a case success rate of almost 100% is demanded and in Bengal a minimum of 75% has been prescribed. Insertion success rate of over 90% is also considered by some.

4. Distribution of vaccine lymph requires the adoption of special measures for maintaining potency of vaccine lymph. The amount of deterioration in potency during transit is not insignificant. The main consideration should be to reduce the time interval between removal from the cold storage and its actual use on children and also to keep it cool during this period, especially during summer months.

4 (i). Two types of vaccine lymph tubes are used at present—glass capillary tubes containing a single dose (three doses in Burma) of vaccine lymph and glass vials with corks containing 20 to 100 or more doses. Tin plated metal collapsible

tubes with pin point orifice and screw caps were popular in some provinces but on account of war they are not available now. Vaccinators are in the habit of using their mouth to blow out vaccine lypmh from capillary tubes. This is obnoxious. In glass vials vaccinators are required to dip the instrument to take out vaccine every time they vaccinate a child. This may affect its bacterial purity. Imported collapsible tubes are very costly. They cannot be reused and, being made of metal, are conductors of heat. Grinding quality or the amount of vaccine lymph in such containers is not visible, because they are opaque. Glass containers are liable to breakage in transit. Single dose capillary tubes are the best but they are costly and sealing of their ends on flame not only takes time but heats up vaccine lymph.

4 (ii). As regards cooling arrangements during transit, packing in moist cotton wool is adopted but it is doubtful if it can be of any real benefit. Use of thermos flasks is not practicable because it increases postal charges and it invariably breaks during transit. Packing in ice is not possible unless the parcels are sent per passenger train which is impossible. Reduction in the time interval between its removal from cold storage and actual use on children seems to be the only satisfactory solution. Vaccinators should try to use the vaccine lymph as soon as it is received.

Sometimes a suggestion is made to have subsidiary distribution centres for vaccine lymph in certain towns where it can be stored in frigidaires, but frigidaire temperature is not cool enough for vaccine lymph and actual trial experiments indicate an appreciable deterioration in potency under such storage.

4 (iii). As regards postal facilities, postal regulations create certain difficulties. They insist on certain type of packing which is not quite convenient and cheap. Up to the last few years vaccine lymph parcels were treated as 'pattern and sample packets' and could be sent at cheap rates but now the postal authorities treat them as regular parcels. This means increased cost on postage and such parcels can no longer be despatched on Sundays and other postal holidays. If they are to be urgently despatched on holidays postal rules require postage at the rates applicable to letters. Leaky parcels are not accepted by postal authorities. This cannot always be avoided as the vaccine lymph containers are made of glass and the containers often leak out during transit. Postal authorities are inclined to treat vaccine lymph as coming under para 83 of Section II of the Post and Telegraph Guide where packing conditions applicable to plague cultures are prescribed. Vaccine lymph does contain living germs but they are of non-pathogenic variety.

4(iv). Definite week days are to be fixed for each vaccinator when he is to attend the post office to take the delivery of the parcel and rigid observance of the despatch programme is required so as to facilitate prompt receipt by vaccinators to avoid dislocation in his programme. In some provinces vaccine lymph is supplied by post to vaccinators directly while in others it is distributed to them through the district medical officers of health. This double posting means delay and doubles the cost of transit. Vaccine should be supplied directly to vaccinators from the vaccine institutes. Indirect supply to vaccinators through the medical officers of health must result in appreciable loss in potency and should be avoided, if possible.

6. Technique of vaccination depends upon the vaccination policy. The present practice is to confer 'maximum' immunity at a single operation irrespective of the severity of reaction. This is why stress is laid on large scar area and

multiple insertions. This probably failed to achieve maximum popularity for vaccination. The so called ' maximum ' immunity afforded by this policy is not sufficient enough to avoid the need of revaccination. So the present tendency is to advocate a vaccination policy which will make vaccination more acceptable and less painful. Multiple insertions and large scar area undoubtedly give better immunity but the necessity of revaccination is not avoided. During an epidemic of smallpox reliance can be placed only on recent vaccination. Recency of vaccination is of greater importance than large scar area produced a few years previously. The general cry is for more ' safe ' lymph. Proper technique of vaccination cannot be discussed unless the vaccination policy is decided. India cannot afford any relaxation which may affect herd immunity. A change in policy may result in less evasions and general rise in the immunity status of the people.

6 (i) Success or failure of vaccination entirely depends upon the way in which the vaccination is done. Vaccinators should be given clear cut instructions regarding handling of vaccine lymph. They carry it in their pockets adjacent to their body. Their body temperature of 37°C not only destroys the potency but incubates extraneous organisms. Metal boxes should not be used by them to keep the vaccine. Wrapping the vaccine tubes in moist cotton wool seems to be the only method available to them. The use of the vaccum flask is out of question, as no ice can be made available in villages. Besides, such flasks may be misused by them for their tea or milk while touring in the villages and not for storing vaccine lymph.

6 (ii) Aseptic precautions adopted by vaccinators require rigid supervision. It is true that inflammatory reaction, noticed, is generally due to infection with pyogenic organisms subsequent to vaccination operation due to carelessness on the part of parents, but it does not mean that aseptic precautions at the time of vaccination operation should not be thorough. Use of soap and water and drying of the arm before vaccination is all that is required. Methylated spirit or alcohol is not considered suitable now. The use of ether or chloroform on the skin is out of the question.

6 (iii). Number, size and depth of insertions are to be determined by the vaccination policy. The usual idea that the depth of an insertion should be such as not to draw blood is no longer held. It should be deep enough to draw some blood but not so deep as to cause free bleeding. As regards the number of insertion, if the policy for less painful vaccination is adopted we should have only two insertions in primary cases. One will not do in India and three or more create unnecessary pain. Two seem to be a good number. If only one insertion is done chances of failure may increase. Case success rate in primary vaccination is about 95% or more. So in primary vaccination if the number of insertions is reduced to two, vaccination may be more acceptable and it may result in general rise in the immunity level of the population. In case of revaccination a single insertion may not be satisfactory. There is some evidence to the effect that multiple insertions result in better ' takes '. Three insertions should be the minimum in case of revaccination. This will not produce any inconvenience because the reaction in revaccination cases is very mild and in some cases it is not noticed at all. In special cases requiring proper judging of the immunity or immediate reaction vaccine lymph may not be applied to one of the three marks which may serve as a control to distinguish it from the traumatic reaction.

Number of insertions and the amount of vaccine lymph (dose) prescribed in various provinces are as follows :—

Punjab.	50 or 60 doses or about 200 insertions per c.c.
Madras.	30 doses or 120 insertions per c.c.
U. P.	25 doses or 75 insertions per c c.
Bombay.	20 doses or 80 insertions per c.c.
C. P.	20 doses or 80 insertions per c.c.
Bengal	2 doses or 8 insertions per grain (primary) 30 doses per c.c.
"	4 doses or 8 insertions per grain (revaccination) 60 doses per c.c.
Bihar.	do.
Assam.	?

As regards the size of insertions rotary lancets are used almost universally in India. So the question of size does not arise. It can be considered only if a change in the type of the instruments used is considered desirable.

6 (iv). Type of instruments used in various countries depend upon the type of insertions advocated. Cross hatching is prohibited. The instruments used in various countries are a needle, lancet drill or a sharp pointed scalpel. The method used is either that of a linear insertion or that of 'multiple pressure' advocated by Leake. In India rotary lancets are used and probably they are as good as any other.

For the application of vaccine lymph to the insertion the handle of the instrument may not be used as it gets contaminated by frequent handling.

6 (v). Care of developing pustule is really the job of parents. No dressing is normally required. All that is really necessary is a sterile gauze secured by zinc oxide strappings applied above and below the site of vaccination. In India this cannot be practicable although protection from dust and flies is to be provided. Keeping vaccination site cool and dry and taking proper care to prevent injury to the crust is all that can possibly be done in rural areas. People apply milk, dew drops, oil or ointment but it has been noticed that all these things delay healing.

6 (vi). Usual inspection of the vaccination result is done after about a week. This is quite satisfactory so far as the primary vaccination is concerned but in case of revaccination if various types of reactions are to be examined two inspections, one 48 hours and the other a week after are essential. Revaccination results recorded at present are not at all reliable. No vaccination is really unsuccessful provided a good lymph is used. It is desirable to record results as 'immediate', 'accelerated' or 'typical primary' vaccinations. Vaccinators require some training on this but it is not difficult for them to grasp. International practice requires the recording of results in the above manner.

5 & 7 (i–v). Basic educational qualification of vaccinators is usually the middle English or the middle Vernacular standard. Practical training in the field is given by senior vaccinators under the supervision of the Medical officers of health. The period of training varies. In some places vaccinators are also trained in general hygiene and first aid. Vaccinators as a rule are the servants of local boards... In some provinces a few government vaccinators have been appointed. Their recruitment is in the hands of medical officers of health. In U. P. a candidate is first appointed as a paid apprentice vaccinator. In Bihar and northern part of Orissa paid vaccinators are employed by the municipalities. In the rural areas they work on contract system during the vaccination season.

Grades of vaccinators vary considerably. Salaries in different provinces are as follows :—

	Asstt. Supdt. of Vaccination.	Vaccinators.
U.P.	I Grade Rs. 60-3-75 II Grade Rs. 40-2-50	Rs. 18-1-35 Paid Apprentice Rs. 15/-
N. W. F. P.	Rs. 40-4-60/5-90	Rs. 18-1-22/2-40
Bombay		I. Rs. 36-1-45 II. Rs. 25-1-35
Assam		Rs. 15-30 (Permanent) Rs. 15-25 (Temporary).
Madras		*Urban.* I. Rs. 30-2/2-50 (men & women) II. Rs. 25-3/2-40 (women) *District Board.* I. Rs. 30-2/2-50 (men & women) II. Rs. 25-3/2-40 (women) *Panchayat Board.* I. Rs. 30-2/2-44-1/2-45 II. Rs. 25-3/2-40 (women)
Baluchistan		Rs. 50/- Urban Rs. 70/- (old) Rural Rs. 30-1-40 (new) Rural.
C. P.		Rs. 18-1-35.
Bengal		Varies from Rs. 10/- to Rs. 20/-.

Supervision of the work of vaccinators is done by the Vaccination Inspectors or Assistant Superintendents of Vaccination, who are under the medical officers of health. In Madras the minimum number of inspections by Medical officers of health has been reduced from 75% in the case of primary and 30% in the case of revaccination to 20% in both the cases. Health Inspectors are required to verify about 40% of the total vaccinations.

Proper supervision of the work of vaccinators is the most important thing. Most of the vaccination deficiencies can be traced to the carelessness or negligence of the vaccinators. Many children remain unprotected. Quality and quantity of work done by vaccinators can improve only if close supervision is exercised. Average number of vaccinations performed by a vaccinator varies considerably in different provinces. It varies from 1200 in Bihar to 7500 in the Punjab. This shows that the average number of vaccinations performed by a vaccinator can considerably be increased if proper supervision is exercised.

The quality and quantity of work can undoubtedly improve considerably if vaccinators do their work efficiently. It has been noticed that if vaccinations are properly performed there is no reason to suspend the work during summer months. Vaccination operations can be performed throughout the 12 months if the vaccine lymph is properly handled. At present vaccination work is done only during the cooler months of the year. In Delhi Province, vaccine lymph has been found to give uniformly good results throughout the year.

A very high percentage of smallpox mortality is noticed in age group under 10. This shows that many people can evade vaccination of their children. Vaccination of children can be performed when they are six months old but the average age of children at the time of vaccination is said to be about 18 months. This undue postponement of vaccination to such a late period causes many to die of smallpox at an early age. This can easily be prevented by close supervision of the work of vaccinators.

(vi). Appointment of female vaccinators is now very desirable. Statistical investigations have been done in certain areas where it is found that the vaccination deficiency in females is proportionately more than in males and that as a result of this specific mortality due to smallpox in females is higher than in males. This can be avoided if female vaccinators are employed. They have been employed in Madras, a few in Karachi city and one in N. W. F. P.

With a view to have proper grasp on the subject of anti-smallpox vaccination in British India some relevant statistical data is given below.

Smallpox Mortality in British India.

	Average annual smallpox deaths.	Mean rate.
1880–84	154,933	0.8
1885–89	79,344	0.4
1890–94	81,447	0.4
1895–99	89,444	0.4
1900–04	88,264	0.4
1905–09	1,11,276	0.5
1910–14	74,790	0 3
1915–19	87,071	0.4
1920–24	56,415	0.2
1925–29	98,051	0.4
1930–34	68,516	0.3
1935–39	67,927	0.3
1940	72,876	0.3
941	59,307	0.2
942	27,137	0.1
943	50,621	0.2

Vaccination Operations and Smallpox mortality in British India.

Year	Number of vaccinations		Percentage of children under one year successfully vaccinated (Primary) to total births during the calendar year.	Smallpox deaths (Calendar year)	Rate
	Primary (all ages)	Revaccination (all ages)			
1931-32	9,012,950	5,334,148	46	37,272	0.1
1932-33	9,365,226	10,082,421	45	44,925	0.2
1933-34	9,284,821	11,604,663	43	1,03,641	0.4
1934-35	9,506,666	12,443,590	44	83,928	0 3
1935-36	1),022,554	14,011,904	45	90,703	0.3
1936-37	10,404,125	12,805,764	45	1,04,805	0.4
1937-38	10,419,989	14,913,397	46	56,180	0 2
1938-39	10,204,648	13,488,906	46	39,844	0.1
1939-40	10,399,676	15,301,134	46	48,103	0.2
1940-41	10,401,614	15,937,083	47	72,876	0.3
1941-42	10,276,227	15,705,531	47	59,307	0.2
1942-43	9,411,394	12,467,155	48	27,137	0.1
1943	..	..	..	50,621	0.2

Percentage of smallpox deaths in children to total smallpox mortality in British India.

	Under one year	Between 1 and 10	Total 0-10
1921	..	..	49.50
1922	..	..	59 03
1923	..	..	59.08
1924	..	..	61.04
1925	..	..	54.40
1926	..	..	48.83
1927	..	..	36.94
1928	..	..	47.80
1929	..	..	57.50
1930	..	..	58.09
1931	..	..	43.1
1932	15	24	39
1933	16	26	42
1934	19	27	46
1935	20	29	49
1936	13	23	36
1937	14	27	41
1938	15.7	27.6	43.3
1939	19.7	30.5	50.2

Sex proportion in vaccination and specific smallpox mortality in Calcutta.

	Vaccination per 1000 of population.		Specific smallpox mortality per 1000 of population.	
	Males	Females	Males	Females
1923	4.6	4.1	0.18	0.20
1924	12.9	10.7	0.24	0.39
1925	25.9	21.4	3.00	5.00
1926	10.9	10.0	0.68	1.00
1927	22.1	20.4	1.90	3.80
1928	6.2	8.6	0.40	0.77
1929	12.5	11.0	0.22	0.40
1930	21.6	27.4	1.20	2.30
1931	23.4	24.2	0.60	1.00
1932	19.2	19.7	0.45	0.83
1933	44.4	50.6	2.70	5.20
1934	22.6	22.0	0.78	1.14
1935	31.8	28.6	0.78	1.40
1936	53.9	52.5	2.80	5.50

Sex proportion in smallpox mortality in Calcutta during smallpox epidemics.

Sex Proportion in population — Males : Females is roughly 2 : 1

	Percentage of male mortality.	Percentage of female mortality.
1936	.. 53.1	.. 46.9
1940–41	.. 56.3	.. 43.7
1944– (Jan.–April)	.. 50.7	.. 49.3
1937–42 (Average) (Excluding 1940–41 epidemic)	.. 51.7	.. 48.3

Smallpox cases in vaccinated persons.

	Total Cases	Vaccinated No.	Vaccinated Percentage.	Unvaccinated No.	Unvaccinated Percentage.
Bombay Fever Hospital (1943–44)	1080	598	55	482	45
Campbell Hospital, Calcutta.	3136	1706	52	1430	48
Bengal ...	758	394	52	365	48
C.P. Adapted from 1935 Report.	194	102	53	92	47
Bombay Adapted from 1935 Report.	258	132	51	126	49
Madras Adapted from 1935 Report.	844	710	84	134	16
Burma Adapted from 1935 Report.	607	355	58	252	42

Average number of vaccinations performed by a vaccinator in various Provinces.

	1938	1939	1940	1941	1942
British India ..	2,809	2,951	..	..	
N. W. F. P. ..	6,294	5,715	5,041	4,983	4,554
Punjab ..	7,720	7,587	6,882	6,858	5,874
Delhi ..	4,658	6,043	4,509	..	..
U.P. ..	1,925	2,219	2,114	2,029	1,962
Bihar ..	1,242	1,520	1,286	1,183	1,324
Orissa ..	2,245	2,436	2,716	3,421	2,875
Bengal ..	3,008	2,772	2,769	3,713	2,659
C. P. ..	1,705	2,175	2,055	1,948	1,790
Bombay ..	2,395	3,200	4,294	4,104	3,145
Sind ..	2,661	2,635	2,274	2,031	1,993
Madras ..	3,025	3,456	5,165	5,070	4,728
Coorg ..	1,001	1,038	2,429	1,732	2,941
Assam ..	1,645	1,833	1,817	..	
Ajmer-Merwara ..	2,140	4,201	1,633	1,234	1,568

INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS

A. (1). *Volume XXXI (1943).—The Journal.*—Volume XXXI coincided with the calendar year and owing to the reduction in the amount of material received for publication, only two half-yearly numbers (May and October) were published. It is intended to limit publication to two numbers a year during the period of war. In the interest of economy and on account of shortage of paper a new style of printing with narrower margins has been adopted resulting in the utilization of 25 per cent more space. The average size for each number was 125 pages of literary and tabular matter with 2 plates, representing a decrease of 39 pages per number as compared with the previous volume.

(2) *The memoirs.*—It has been decided to suspend the publication of *Memoirs* during the war except for work likely to aid the war effort and as no such material was received, no *Memoir* was published with this Volume.

(3) *The net cost of Volume XXXI* (2 numbers only) was Rs. 3,402.

B. (1). *Volume XXXII (1944).—The Journal.*—During the year which has elapsed since last meeting only 1 number (May 1944) has been published and this appeared in July 1944. The slight delay was due to late submission of papers to the Printers. Publication of the second (October 1944) number has been delayed and this will not appear until about the middle of December. The delay has been caused chiefly by the Editor's absence on tour. The May issue contains 108 pages and 2 plates, while the October number will consist of approximately 142 pages of print with 4 plates, thus the avergae for the two numbers of the volume will be 125 pages with 3 plates, the size being the same as for the previous volume.

(2) *The Memoirs.*—No *Memoir* has been published with this Volume and no material for one has so far been received.

C. *Distribution;*

Indian subscribers	117	= 211	Same as for 1943 as figures for 1944 are not yet available.
Foreign subscribers	94		
Exchange list	186	= 310	Less 72 suspended owing to war = 238.
Free list	124		
Voucher copies to advertisers, regn., make-up, police, specimen, propaganda, etc.	40 approx.		
	561 or 489		
Balance left with Publishers for sale ..	139 or 211		
Total copies printed..	700		

The Exchange and Free lists have been increased by 6 over last year by addition of new addresses. About 75 foreign addresses on the Distribution lists were suspended owing to the war but 3 of these have since been resumed. There has thus been a total increase of 9 in the distribution as compared with last year. A large number of publications are obatined in exchange for the I. J. M. R. for the I. R. F. A. Library, though some from enemy countries have been suspended.

D. *Distribution lists.*—These were last revised in July 1938. As there will be many changes and additions, it is proposed to revise this list again to bring it up to date soon after the war is over and normal times prevail.

E. *Number of copies printed.*—From July 1940 it has been decided to print only 700 copies instead of 900, as this number is considered sufficient, according to former experience, to meet all demands. Volumes I to VIII are out of print.

F. *Cost of printing the journal.*—The following statement shows the cost of production during the last five years :—

Cost of printing the Journal.

Number.	XXVI–XXVII, 1939–40.	XXVII–XXVIII–XXIX, 1940–41.	XXIX XXX, 1941–42.	XXX, 1942.	XXXI, 1943.
April ..	3,272	5,137	2,052 *	2,949 *	May–2,633
July ..	4,212	3,932 *	3,531	1,650	
October ..	4,611	4,717	3,413	2,413	Oct.–2,486
January ..	3,640	3,571	3,531	Not published.	
Average pages	260	317	214	158	125
Totals ..	15,739	17,357	11,527	7,012	5,199

* 700 copies only.

Recoveries.

April ..	1,628	1,452	1,325	1,034	May–837
July ..	1,628	1,451	1,325	946	
October ..	838	963	837	1,518	Oct.–880
January ..	838	963	837	—	
Totals ..	4,932	4,829	4,324	3,498	1,717

G. *Net cost of the Journal and Memoirs.*—The following statement shows the net cost of the production of the *Journal* and *Memoirs* during the last five years :—

Journal.

April	1,648	3,685	727	1,915	May–1,796
July ..	2,584	2,481	2,206	704	
October ..	3,773	3,754	2,576	895	Oct.–1,606
January ..	2,802	2,608	1,694	—	
Totals ..	10,807	12,528	7,203	3,514	3,402

Memoirs.

No. 31 1,754	..	No. 33 1,565	..	..
No. 32 1,632	..	No. 34 1,627	..	..
Totals ..	3,386	..	3,192	..	..
Grand totals.	14,193	12,528	10,395	3,514	3,402

For convenience of accounting all receipts are credited to the *Journal* account and the figures given for the *Memoirs* are the actual cost of production.

The net cost during 1943 after deduction of receipts was Rs. 3,402. All Publishers' bills for 1943 were submitted in time to make payments within the financial year.

H. *Financial position for the year 1944-45.*—The budget allotment for the year was Rs. 7,000 for the cost of 2 half-yearly numbers of the *Journal* and Rs. 2,000 for any *Memoir* which might be published. The following is the estimated expenditure :—

Vol. XXXII,	No. 1 (May 1944) and No. 2 (Oct. 1944) @ Rs. 3,500	Rs. 7,000 estimated.
No *Memoir* was published	..	Nil
	Total ..	Rs. 7,000

The saving anticipated is Rs. 2,000.

J. *General.*—Publication of the *Journal* has been continued on the lines laid down at the Conference of 1932. The services of the members of the Editorial Board have been fully utilized. The Editor has put forward proposals for revising editorial policy and for enlarging the Editorial Board. These proposals are under consideration at present.

Papers submitted from other countries are generally refused unless the subject is of direct Indian interest. Only paper accepted from outside India was on malignant tumours in Ceylon (Cooray, May 1944).

The efforts made to keep the *Journal* at the minimum size for reasons of economy in paper have resulted in a considerable reduction in size as compared with the previous years. Many contributors have cooperated in this and have made reasonable reductions in their papers when asked to do so.

In Vol. XXXII (May and Oct. 1944) out of 39 articles published 14 were on nutrition, 10 on bacteriology, 4 on biochemistry, 1 on pharmacology and 2 on haematology.

K. *Budget estimate for 1945-46.*—It is intended to continue publishing only two numbers of the *Journal* during 1945 which will be produced in May and October. The following is the estimated cost of publication during the year for 700 copies of 2 numbers and 1 *Memoir* which may be received :—

2 half yearly issues of the *Journal* @ Rs. 3,500	..	Rs. 7,000
1 *Memoir*	..	Rs. 2,000
Total	..	Rs. 9,000

The annual subscription has been reduced from Rs. 16 to Rs. 8, while the cost of individual single numbers remains as before.

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1944.

A. Indian Journal of Medical Research and Indian Medical Research Memoirs.

1. AHUJA, M. L., and BROOKS, A. G.—In vitro Test for Assay of Cobra Antivenenec (Vol. 32, No. 2).
2. BANERJEA, R.—Some Observations on Voges-Proskauer Test (Vol. 32 No. 1).
3. BHATIA, B. B., and KAPUR, R. D.—Pharmacological Action of Alkaloids of *R. serpentina* Benth. Part I. Neo-ajmaline and Iso-ajmaline (Vol. 32, No. 2).
4. BOSE, A. N., and GHOSH, J. K.—Studies on Sulphanilyl-benzamide : Toxicity and Absorption (Vol. 32, No. 1).
5. BOSE, B. C., and AHUJA, M. L.—Detection of Pyrogens in Fluids by Biological Methods (Vol. 32, No. 1).
6. BOSE, B. C., and MUKERJI, B.—Use of Trichlorethylene as an Anaesthetic Agent : An Experimental Study on Laboratory Animals (Vol. 32, No. 1)
7. DAY, C. D. MARSHALL, and SHOURIE, K. L.—The Incidence of Periodontal Disease in the Punjab (Vol. 32, No. 1).
8. DHARMENDRA, and MUKHERJI, N.—Attempts to Transmit Human Leprosy to Splenectomized Monkeys (Vol. 32, No. 2).
9. DHARMENDRA, and MUKHERJI, N.—The Effect of Sulphapyridine on Experimental Rat Leprosy (Vol. 32, No. 2).
10. GREVAL, S. D. S., and BHATTACHARJI, J. N.—Some Physical and Immunological Differences between Natural and Re-constituted Human Serum (Vol. 32, No. 1).
11. GUPTA, J. C., and KAHALI, B. S.—General Pharmacology of *Umbellatine*, a new Alkaloid isolated from *Berberis umbellata* Wall and *Berberis insignis* Hook. f. and its Use in the Treatment of Oriental Sore (Vol. 32, No. 1).
12. GUPTA, J. C., KAHALI, B. S., and DUTT, A.—The hypnotic effect of a resin fraction isolated from root of *rauwolfia serpentina* Benth. obtained from Dehra Dun (Vol. 32, No. 2).
13. KULKARNI, R. N.—Quantitative estimation of common Trihalogen volatile anaesthetics in blood and tissues of animals (Vol. 32, No. 2).

14. KAMALA BHAGWAT, and DEVI, P.—Anti-Thiamine factor in Carp (Vol. 32, No. 2).

15. KAMALA BHAGWAT, and DEVI, P.—Inactivation of Thiamine by certain foodstuffs and oil seeds. Part I. Estimated by Chemical Methods (Vol. 32, No. 2.)

16. KAMALA BHAGWAT, and DEVI, P.—Inactivation of Thiamine by certain foodstuffs and oil seeds. Part II. Estimated by Biological Methods (Vol. 32, No. 2).

17. KAMALA BHAGWAT, and SREERAMAMURTHY, V. V.—Liberation of Tyrosene, Tryptophane, Cystine and Arginine from Proteins (Vol. 32, No. 2).

18. KIRWAN, E. O'G., SEN, K., and BOSE, N.—Vitamin B_2 deficiency as a cause of eye diseases in Bengal (Vol. 32, No. 2).

19. MAJUMDER, D. N., and DAS GUPTA, C. R.—Haematological studies in *Silenus* (*Macacus*) *rhesus*. Part I. The blood picture of the normal monkey (Vol. 32, No. 2).

20. MASON, ELEANOR D.—Daily Measurements of Basal Metabolism, body temperature and pulse rate during a journey to the Tropics (Vol. 32, No. 1).

21. PATWARDHAN, V. N., CHITRE, R. G., and SUKHATANKAR, D. R.—Studies in Calcium and Phosphorus Metabolism. Part VI. The Ionic products of Calcium Phosphates in blood serum of rachitic infants (Vol. 32, No. 1).

22. RANGANATHAN, S.—Studies on experimental Flourine poisoning in rats (Vol. 32, No. 2)

23. RAO, S. DATTATREYA.—Studies on destruction of Vitamin A in Shark-Liver Oil. Part. I. Destruction during normal storage and standardization of Pulfrich Photometer for the estimation of Vitamin A (Vol. 32, No. 2).

24. RAO, S. DATTATREYA, and BANERJEE, B. N.—Studies on the destruction of Vitamin A in Shark Liver Oil. Part II. Development of acidity during storage of livers (Vol. 32, No. 2).

25. RAY, N., and BASU, U. P.—Influence of Phenols on Phenol Reagent (Vol. 32, No. 1).

26. REDDY, D. GOVINDA, and RAO, S. V. RAJA—Blood and bone-marrow in Pre-cirrhotic Toxic Splenomegaly due to Manganese (Vol. 32, No. 1).

27. REDDY, D. V. S., and SASTRY, P. B.—Studies in Vital Capacity (Vol. 32 No. 2).

28. SARMA, P. S.—The estimation of Pyridoxine (Vitamin B_6) in foods using Rice-moth Larvae (*Corcyra cephalonica* St.) (Vol. 32, No. 2).

29. SARMA, P. S.—Egg-white Injury (induced Biotin Deficiency) in Rice-moth Larvae (*Corcyra cephalonica* St.) (Vol. 32, No. 2).

30. SEN, S. N., and BASU, P. N.—Enhancement of Diphtheria Toxin production by Glycolysis (Vol. 32, No. 1).

31. SETNA, S. B., SARANGDHAR, P. N., and GANPULE, N. V.—Nutritive values of some marine fishes of Bombay (Vol. 32, No. 2).

32. SIDDIQUI, M. A. H., and SHAH, M. A.—Estimation of stature from long bones of Punjabis (Vol. 32, No. 1).

33. SWAMINATHAN, M.—Nicotinic acid content of Indian foodstuffs (Vol. 32, No. 1.)

34. VEERARAGHAVAN, N.—A Protozoan Parasite of the Central Nervous System (Vol. 32, No. 2).

B. *Journal of the Malaria Institute of India.*

1. COVELL, G.—Notes on the distribution, breeding places, adult habits and relation to malaria of the anopheline mosquitoes of India and the Far East (Vol. V, No. 4).
2. SIDDONS, L. B.—The experimental transmission of Quartan Malaria by *A. culicifacies* Giles (Vol. V, No. 3).
3. SIDDONS, L. B.—Observations on the influence of atmospheric temperature and humidity on the infectivity of *A. culicifacies* Giles. (Vol. V, No. 3)
4. YACOB, M., and SWAROOP, S.—" The forecasting of epidemic malaria in the Punjab " (Vol. 5, 3, June 1944).

C. *List of papers published by workers under the I.R.F.A. in Journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

AYKROYD, W. R.—Nutrition (Oxford Pamphlets on Indian Affairs, No. 21, May, 1944).

AYKROYD, W. R.—An early reference to night-blindness in India and its relation to diet deficiency (Current Science, 1944, Vol. 13, pp. 149-150).

BHAGVAT, K.—Microbiological assay for pantothenic acid (Current Science, 1944, Vol. 13, pp. 75-76).

BHAGVAT, K. & NIRANJAN SINGH SEKHON.—Nutritional requirements of—*L. Gulgaricus*, *L. Acidophilus* and *Streptococcus Lactis* (Current Science, 1944, Vol. 13, pp. 45-46).

COVELL, G.—Note on malaria situation in Bengal. (Ind. *Health* Gaz. No. 1).

DAS GUPTA, C. R.—A preliminary note on the preparation of anti - Rh agglutinin (I.M.G. Vol. LXXIX, No. 8, Aug. 1944, p. 372).

DEY, N. C. & GHOSH, L. M.—Studies of Ringworm Part I. (Microsporum Audonini infection in India, I.M.G. Vol. LXXIX, No. 2 February, 1944, p. 51).

DHARMENDRA.—Intradermal tests with antigens prepared from the urine of cases of leprosy (*Leprosy in India*, Vol. XVI, No. 2).

DHARMENDRA & SANTRA, I.—The use of iodised hydnocarpus oil in the treatment of leprosy (*Leprosy in India*, Vol. XVI, No. 2).

GUPTA, J. C. & GANGULI, S. C.—The effect of quinine and stilbamidine (M. & B. 744) on the Reticulo-endothelial system as measured by the Congo-red index (I.M.G. Vol. LXXIX, No. 3, March, 1944).

GUPTA, J.C.—' Lipoid nephrosis ' cured after an attack of typhoid fever (I.M.G. Vol. LXXIX, No. 5, May 1944).

JASWANT SINGH & BHATTACHARJI, L.M.—Rapid staining of malarial parasites by a water soluble stain (I.M.G. Vol. 79. No. 3).

PANJA, D. & GHOSH, L.M.—Report on the occurrence of Naga sore in Calcutta (I.M.G. Vol. LXXIX, No. 1. January. p. 17).

PAUL, B.M. & CHATTERJEE, B.C.—Pyrogenic reactions following intravenous saline infusions (I.M.G. Vol. 79, No. 7, July, 1944 p. 305).

VERMA, O.P.—Note on the treatment of angular conjunctivitis Riboflavin (I.M.G. Vol. LXXIX, p. 258).

YACOB, M. & SWAROOP, S.—Degree of accuracy realized in malaria forecasts in the Punjab for the years 1923 to 1942 (I.M.G. Vol. LXXIX, No. 6, June, 1944).

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 28th and 29th November, 1944.

	Enquiries.	Budget allotments Rs.
1.	Cholera treatment units for evaluating the efficacy of sulphaguanidine in the treatment of cholera at the School of Tropical Medicine, Calcutta, and at the King Institute, Guindy, Madras. The Directors of the School of Tropical Medicine, Calcutta, and the King Institute, Guindy, Madras, were asked kindly to submit detailed proposals for the necessary staff etc. which they would require for the units.	
2.	Enquiry to prepare monospecific high titre cholera sera to facilitate accurate identification of strains in various parts of India at the King Institute, Guindy, Madras. The Director, King Institute, Guindy, Madras was asked to submit a detailed budget to carry out this enquiry.	
3.	Malaria Institute of India, Research Section, Delhi. ..	38,539
4.	Southern India Branch of the Research Section of the Malaria Institute of India at the Pasteur Institute, Coonoor. ..	13,862
5.	Statistical enquiry into the methods of forecasting epidemic malaria under Khan Bahadur Dr. M. Yacob, Assistant Director of Public Health, Punjab, and Mr. Satya Swaroop, Statistical Officer, Public Health Department, Punjab, Lahore. ..	2,377
6.	Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.	21,233
7.	Nutrition Research Laboratories, Coonoor.	86,721
8.	Additional nutrition enquiry, (a) Assistant Director, Nutrition Research Laboratories, I.R.F.A., Coonoor, (b) three field workers and (c) enquiry on beri-beri in Cocanada. (*The cost to be met form the balance of Rs.* 30,000/- *annual grant sanctioned by the Governing Body over a period of five years out of the special ten lakhs grant made by Government for anti-malaria and nutrition work.*)	27,961
9.	Ascorbic acid enquiry under Dr. B.N. Ghosh at the University College of Science and Technology, Calcutta.	6,120
10.	Enquiry on the formation, functions and variations of plasma proteins in health and disease under Mr. N.C. Datta at the Grant Medical College, Bombay	2,224
11.	Enquiry into factors affecting normal calcium metabolism in certain areas in the Punjab under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore. ..	3,269
12.	Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipore, Patna	2,355

	Enquiries.	Budget allotments Rs.
13.	Enquiry into the relationship between vitamin B complex and tumor growth under Dr. V.R. Khanolkar at the Tata Memorial Hospital, Bombay. The Board recommended that this enquiry should be absorbed into the clinical research unit under Dr. V.R. Khanolkar at the Tata Memorial Hospital, Bombay, which is to be established from the lst April, 1944. (*See item* 37).	
14.	Nutrition research unit under Dr. K. P. Basu at the Dacca University, Dacca.	15,815
15.	Nutrition research unit under Professor S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.	16,083
16.	Enquiry into methods of treating sick starving destitutes in Calcutta based on the All-India Institute of Hygiene and Public Health, Calcutta.	18,656
17.	Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Punjab University Institute of Chemistry, Lahore.	2.562
18.	Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.	7,838
19.	Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.	4,067
20.	Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras.	3,337
21.	Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.	11,727
22.	Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras. ..	2,382
23.	Plague researches under the Director, Haffkine Institute, Parel, Bombay.	11,754
24.	Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.	10,897
25.	Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra at the Drug Research Laboratories, Jammu, Kashmir	5,046
26.	Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.	4,130
27.	Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.	7,054
28.	Enquiry entitled, "Studies in tests of physical fitness in Indians" under Dr. D. M. Telang at the Grant Medical College, Bombay.	2,107

	Enquiries.	Budget allotments Rs.
29.	Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta.	5,832
30.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras.	6,404
31.	Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay. ..	4,514
32.	Enquiry on the action of snake venoms and their constituents on the bio-chemical activity of tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.	3,116
33.	Enquiry on typhus under Dr. S. R. Savoor at the Haffkine Institute, Bombay. (* This includes a sum of Rs. 11,220 for the pay and allowances of Dr. Savoor).	*23,735
34.	Enquiry into vaccine lymph under the Director of Public Health, Bombay.	200
35.	Enquiry into Naga sore under the Director, Pasteur Institute, Shillong.	10,400
36.	Enquiry into the fraction of liver most suitable for the treatment of tropical macrocytic anaemia under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta. ..	6,216
37.	Clinical research unit under Dr. V. R. Khanolkar, Director of Laboratories, Tata Memorial Hospital, Bombay.	9,502
38.	Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relations of dietary protein to blood and muscle haemoglobin under Professor M. Damodaran, Director, University Biochemical Laboratory, Triplicane, Madras.	3,228
39.	Enquiry on serum standardisation under the Director Central Research Institute, Kasauli.	10,272
40.	Enquiry on kala-azar under Dr. P. C. Sen Gupta, Officer-in-Charge of the Kala-azar Department at the School of Tropical Medicine, Calcutta.	5,408
41.	Enquiries on (i) the value of despeciated bovine serum as a transfusion material in the treatment of inanition and hypoproteinaemia due to starvation and other conditions involving transfusion treatment (ii) other studies in connection with starvation and nutrition—(a) a study of hepatomegaly and liver cirrhosis in relation to starvation and hypoproteinaemia (b) studies of parasprue, its aetiology, diagnosis and treatment (iii) studies of haemoglobinurias seen in India by investigation of the blood pigments under Dr. John Lowe, Professor of Tropical Medicine at the School of Tropical Medicine, Calcutta.	3,792
42.	Enquiry on chronic ulcers in Vizagapatam under Dr. R. Mahadevan, Professor of Operative Surgery, Andhra Medical College, Vizagapatam.	2,670

	Enquiries.	Budget allotments Rs.
43.	Enquiry on hill diarrhoea under Dr. G. Panja, Professor of Pathology and Bacteriology at the School of Tropical Medicine, Calcutta.	1,600
44.	Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.	2,200
45.	Enquiry into infant and child morbidity and mortality in Calcutta under Dr. J. Orkney, W.M.S., Professor of Maternity and Child Welfare, All-India Institute of Hygiene and Public Health, Calcutta.	11,316
46.	Enquiry into anaemia in pregnancy in the Singur Health Unit under Dr. J. Orkney, W.M.S., Professor of Maternity and Child Welfare, All-India Institute of Hygiene and Public Health, Calcutta.	7,140
	GRANTS.	
(i)	Administration charges	41,205
(ii)	Contribution towards the cost of two professorships at the School of Tropical Medicine, Calcutta.	29,035
(iii)	Contribution towards the cost of the plague enquiry in the Salem District under the Government of Madras. ..	3,000
(iv)	Contribution to the Imperial Institute of Entomology, London. (£ 100/-)	1,350
(v)	Contribution to the Bureau of Hygiene and Tropical Diseases, London (£ 150/-)	2,025
(vi)	Contribution towards the cost of the Statistical Bureau in the office of the Public Health Commissioner with the Government of India.	2,003
(vii)	One research fellowship of the value of Rs. 150/-. ..	1,800
(viii)	Indian Journal of Medical Research and Memoirs ..	
	(a) Publication charges of the Journal .. 7,000	
	(b) Publication charges of the Memoirs .. 2,000	
	(c) Editor's office 4,944	13,944
(ix)	I.R.F.A. Library at the Central Research Institute, Kasauli.	2,118
(x)	Pay (including special pay, overseas pay and allowances) and contributions towards passage, leave salary and pension or contributory provident fund of the officers of the Medical Research Department under the Association.	1,50,000
(xi)	Travelling allowance and certain contingent expenses for Medical Research Department Officers employed under the I.R.F.A. and attached to Central or Provincial laboratories.	2,000
(xii)	Pay and travelling allowance of the two Junior Research Workers.	16,675

	Enquiries.	Budget allotments Rs.
(xiii)	Clerical assistance at the School of Tropical Medicine, Calcutta.	3,822
(xiv)	Clerical assistance at the All-India Institute of Hygiene and Public Health, Calcutta.	1,416
(xv)	Travelling allowance for subordinate I.R.F.A. workers on transfer	1,500
(xvi)	I.R.F.A. contribution and interest towards the Contributory Provident Fund	13,000
(xvii)	Miscellaneous unforeseen expenditure	15,000
(xviii)	Imprest advances for enquiries	9,995

00134335

Digitized with financial assistance from

Government of Maharashtra

on 01 February, 2020